A Light for Science

ESRF

European Synchrotron Radiation Facility

A Light for Science

Optimised Data Collection Strategies

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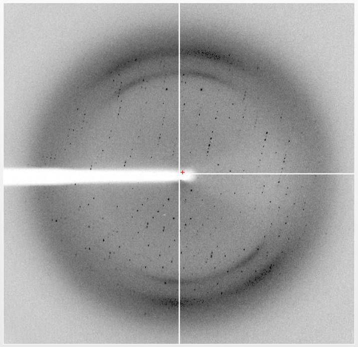
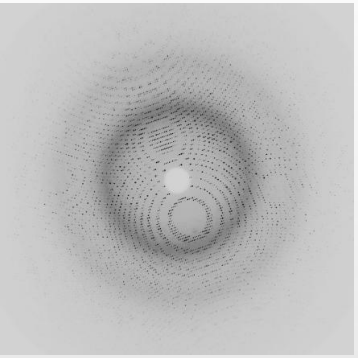
ESRF, MX group

European Synchrotron Radiation Facility

The purpose of a crystallographic data collection is to extract the required structural information from a crystal given finite available experiment time and the limited crystal lifetime in an X-ray beam

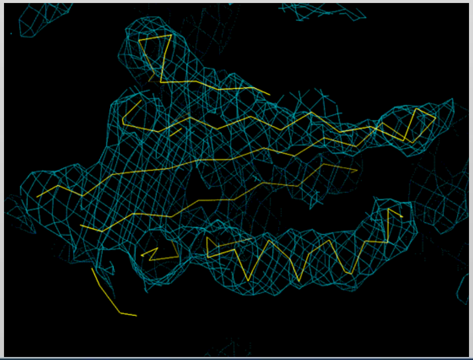


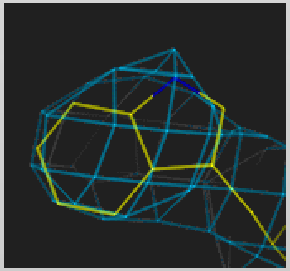


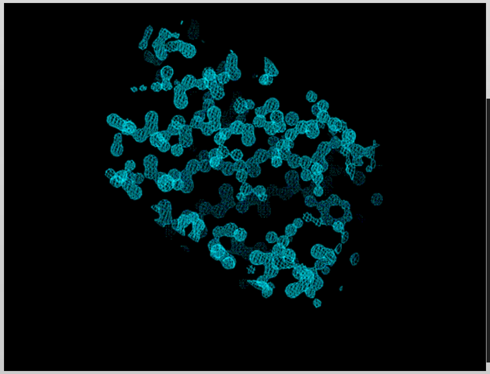
Data quality

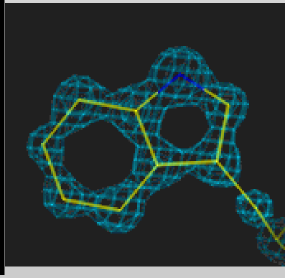
Completeness ***Resolution*** ***Statistics***

Good-quality data will always make structure solution easier and will produce more faithful electron density as well as a more accurate atomic model. It is therefore important to carry out the diffraction experiment under optimal conditions





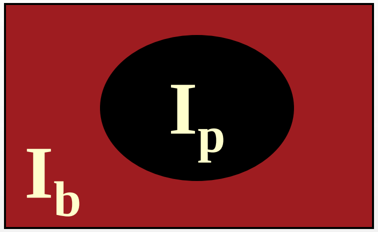




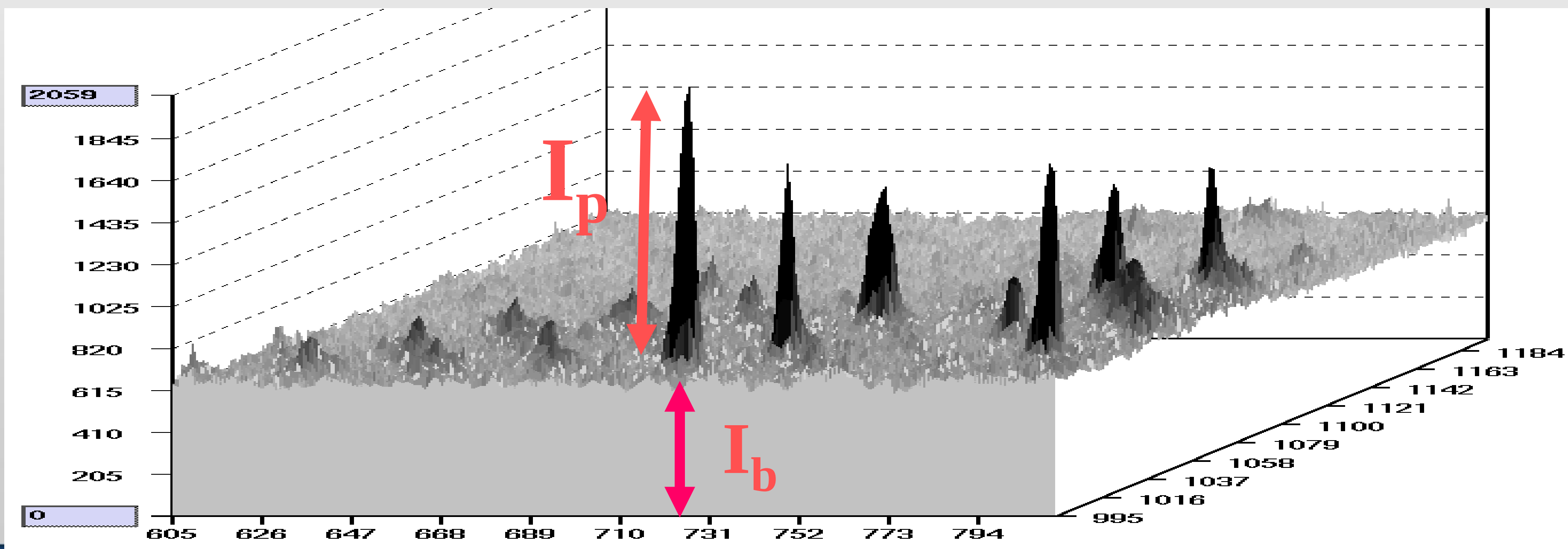
Accuracy

Main uncertainties of the observed intensities are determined by counting statistics

$$\sigma_{I_p}^2 = \left(I_p + I_b \cdot \frac{m \cdot (m+n)}{n} \right) \cdot G$$

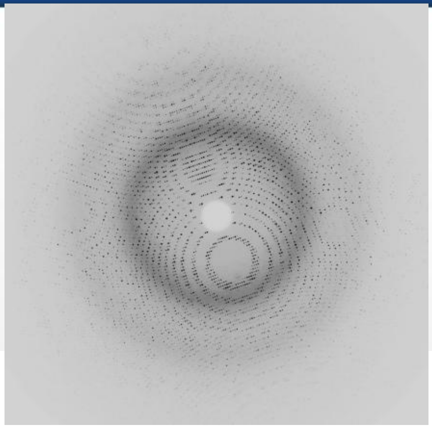


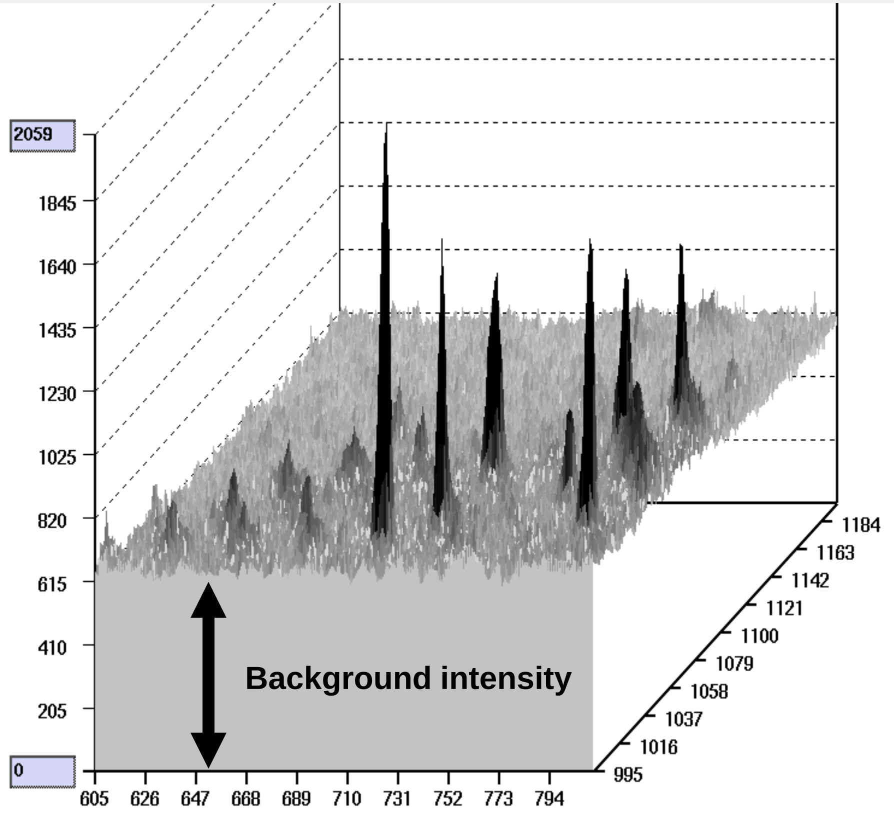
where *m* and *n* are number of pixels in the peak and background region of the measurement box respectively. *G* is the detector gain, which converts pixel counts to equivalent X-ray photons. K_{ins} is a proportionality constant for the instrument-error term

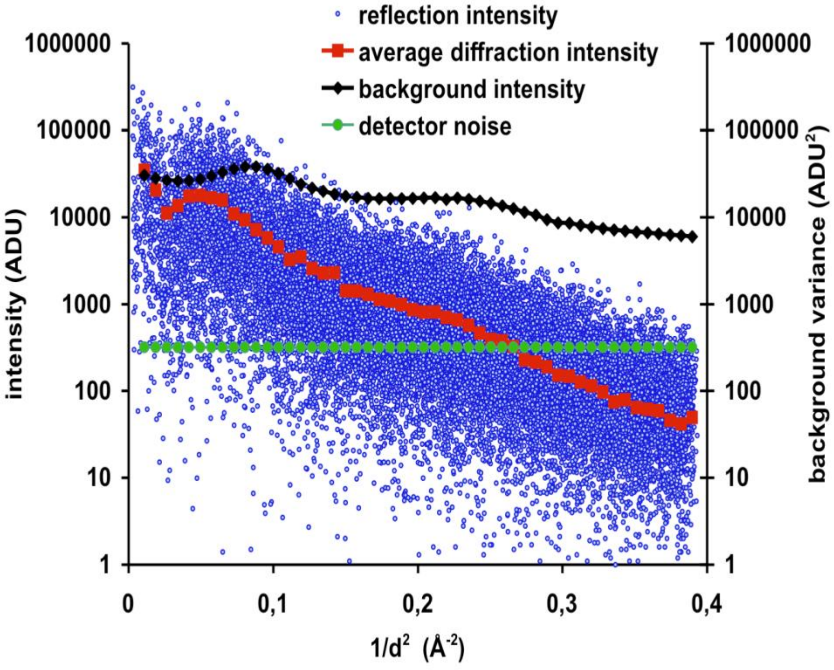


Complications

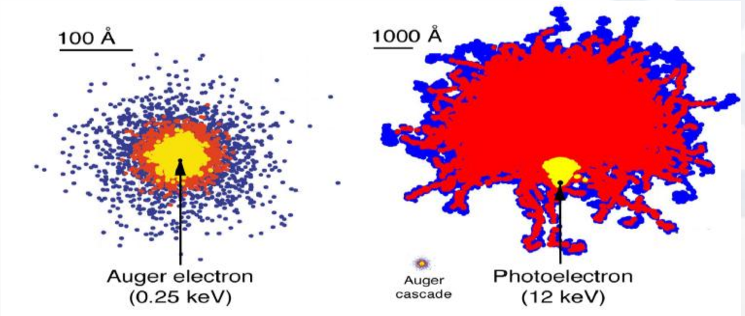
- **Large cell parameters**
- **Weak diffraction intensity – light atoms**
- **Poor crystal quality – big B- factor**
- **Background intensity > diffraction intensity**





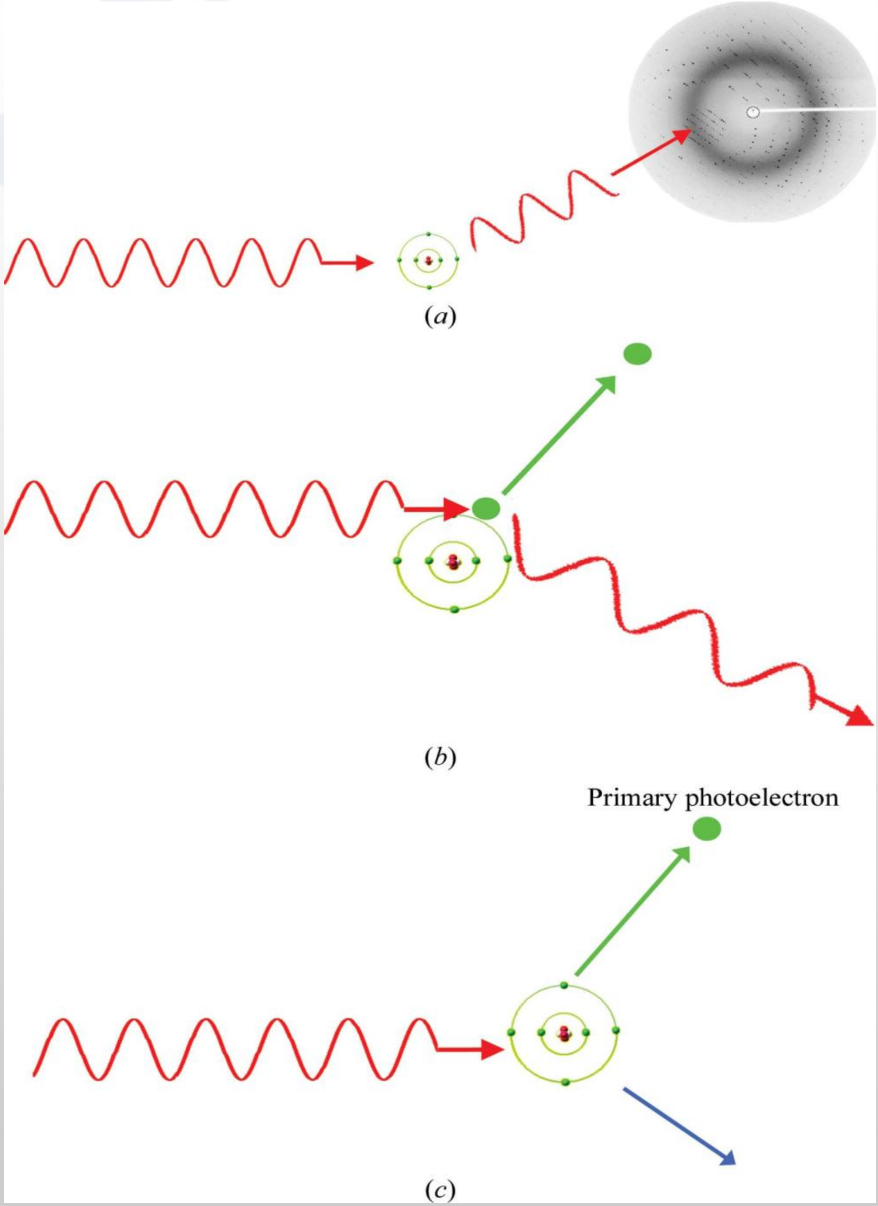


Photon interaction with soft matter



Ziaja et. al, 2000-2002

Primary X-ray interaction processes with the atoms of the crystal and solvent. (a) Elastic (Thomson, coherent) scattering. (b) Compton (incoherent) scattering. Energy is lost in the crystal, contributing to the absorbed dose. (c) Photoelectric absorption. The X-ray transfers all its energy to an atomic electron, which is then ejected and can give rise to the ionization of up to 500 other atoms. The excited atom can then emit a characteristic X-ray or an Auger electron to return to its ground state. (Garman Acta Cryst. (2010). D66, 339–351)



DOSE
Absorbed Energy per Unit of Mass
Gy = 1 J/Kg

Defined by :

- beam energy
- flux density
- atomic composition of the sample
- absorption cross sections

Variation with composition:

- +-50% depending on solvent content, salt, ions, sequence (Cys , Met, SeMet)
- up to factor ~3 for heavy atom soaks at peak absorption

RADDOSE (Murray, Garman, Ravelli)

Henderson limit - 2 10^7 Gy

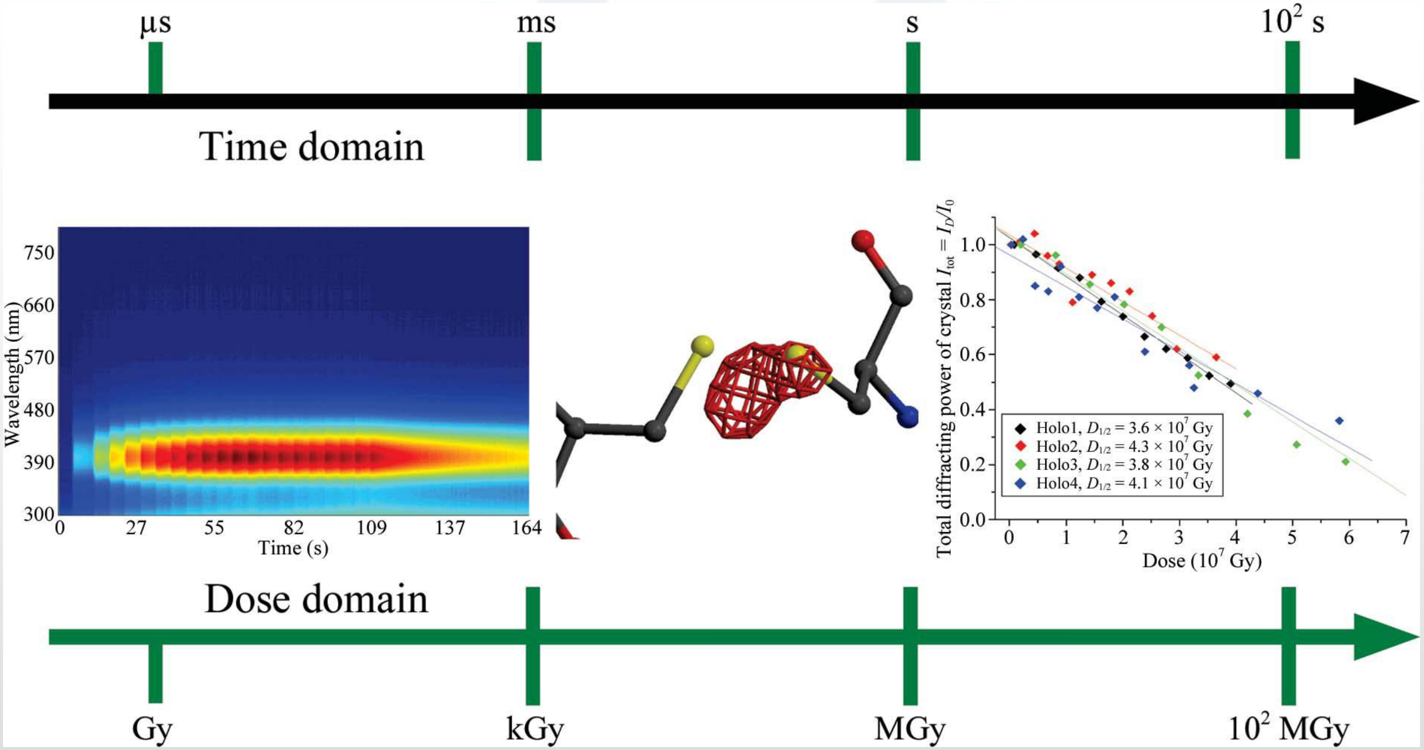
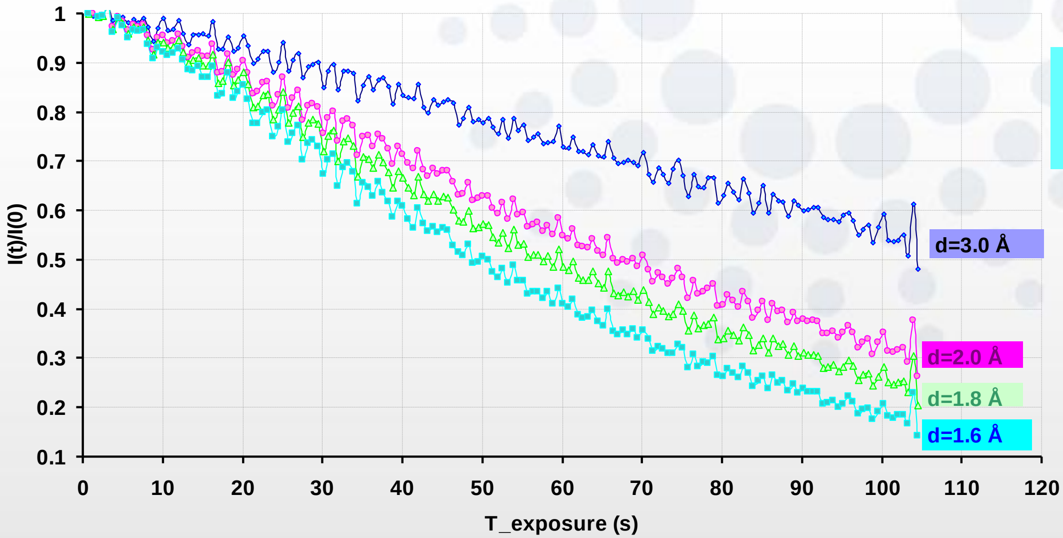


Illustration of radiation damage over a wide range of time scales and dose. Left, UV–vis absorption spectrum of a cryocooled solution of cysteine, showing an intense peak at 400 nm corresponding to disulfide-anion radical production. (Southworth-Davies & Garman, 2007).

Garman, Acta Cryst. (2010). D66, 339–351

Non-specific Radiation Damage
Diffraction Intensity is a function of dose



Oxoanion polyreductase
ID29 ESRF

Kmetko et al. (2006)

Acta Crystallographica Section D
Biological
Crystallography
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Quantifying X-ray radiation damage in protein crystals at cryogenic temperatures

Jan Kmetko,*‡ Naji S. Husseini, Matthew Naides, Yevgeniy Kalinin and Robert E. Thorne

The dependence of radiation damage to protein crystals at cryogenic temperatures upon the X-ray absorption cross-section of the crystal has been examined. Lysozyme crystals containing varying heavy-atom concentrations were irradiated

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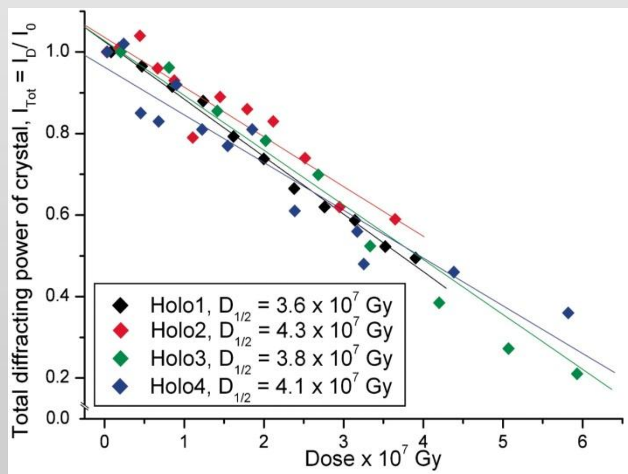
Table 2
Coefficients of sensitivity, $s_{AD} = (\Delta\langle u^2\rangle/\Delta D) = (\Delta B_{rel}/8\pi^2\Delta D)$, for various proteins as determined from dose curves.

Protein	Lysozyme	Catalase	Thaumatin	Apoferritin
PDB code†	1lz8	8cat	1ly0	1ier
Space group	$P4_32_12$	$P3_221$	$P4_12_12$	$F432$
MW (kDa)	14.4	230	22.2	476
Solvent content‡ (%)	39	53	56	61
Photon energy (keV)	8.82	10.0	9.26	9.66
Mass-energy absorption coefficient μ_{en}/ρ ($cm^2 g^{-1}$)	8.3	4.3	6.0	7.0
Coefficient of sensitivity s_{AD} ($Å^2 MGy^{-1}$)	0.012	0.012	0.018	0.017

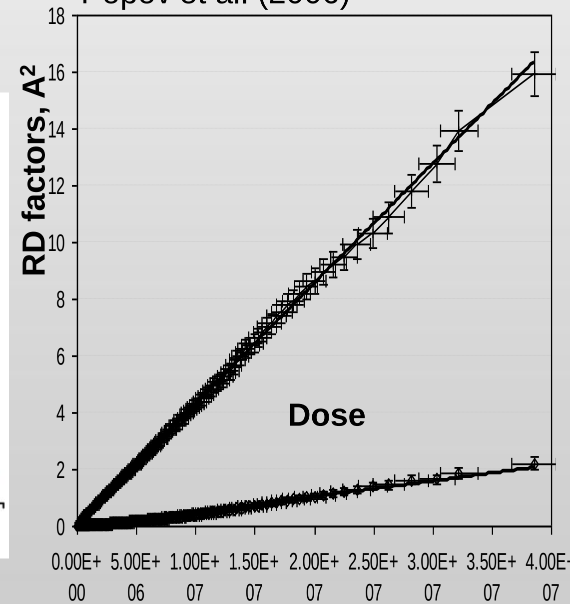
$\beta = 8\pi^2 s_{AD}$ = 0.95 0.95 1.4 1.3 $Å^2$

Owen et al. (2006)

$D_{½}$ = 4.3(±0.3) x10^7Gy



Popov et al. (2006)

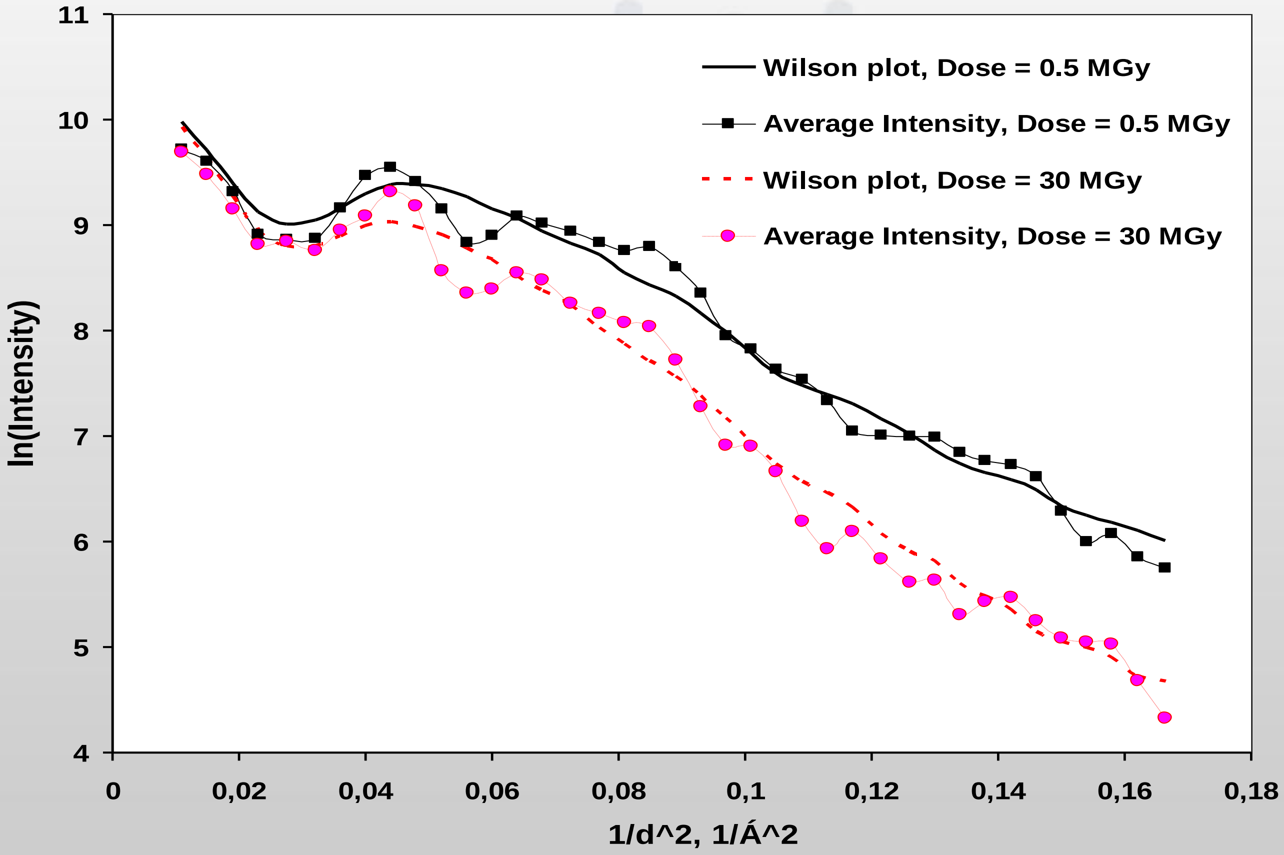


Radiation Damage Modele

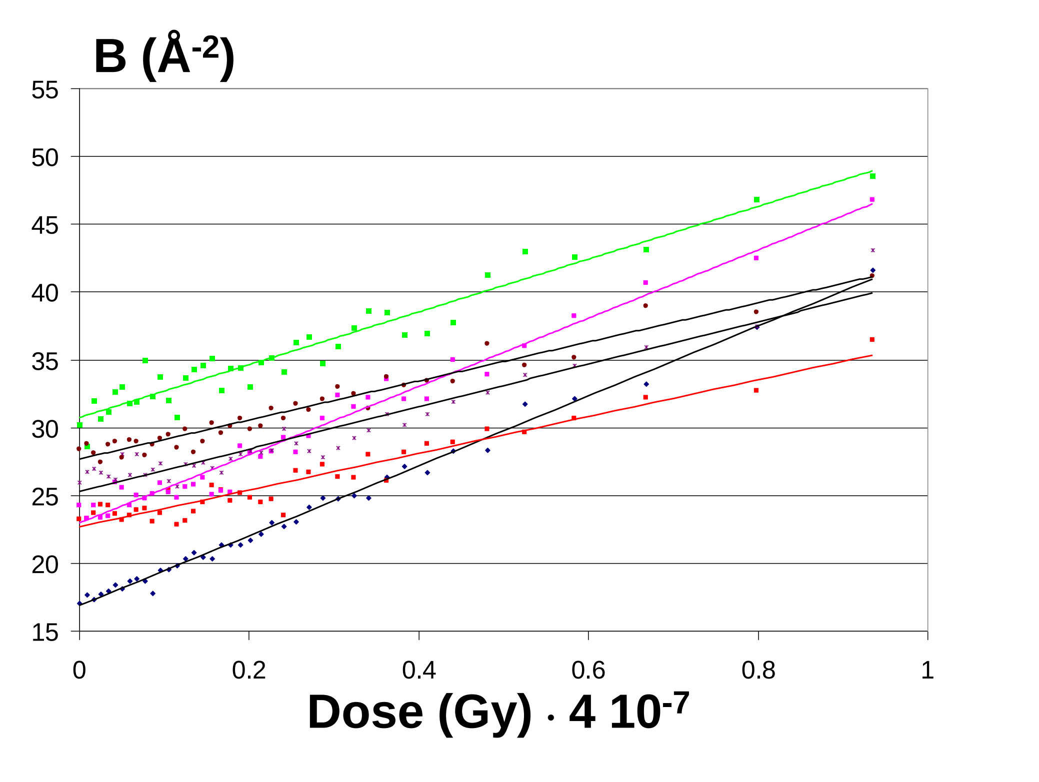
Resolution-dependent intensity decay

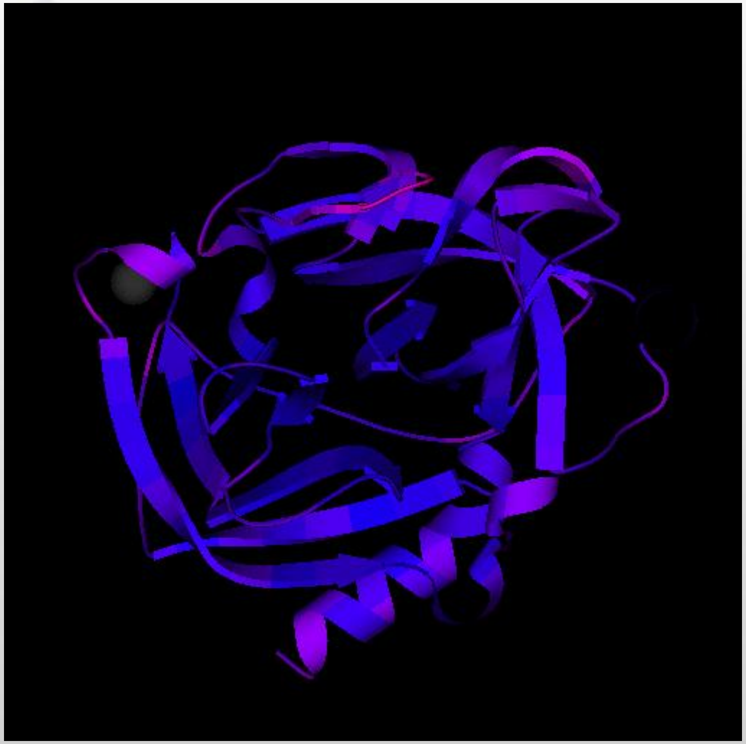
$$\hat{J}(\mathbf{h}, D) = \hat{J}(\mathbf{h}, D = 0) scale(D) \exp(-B(D)h^2 / 2)$$

$$B(D) = \beta D$$

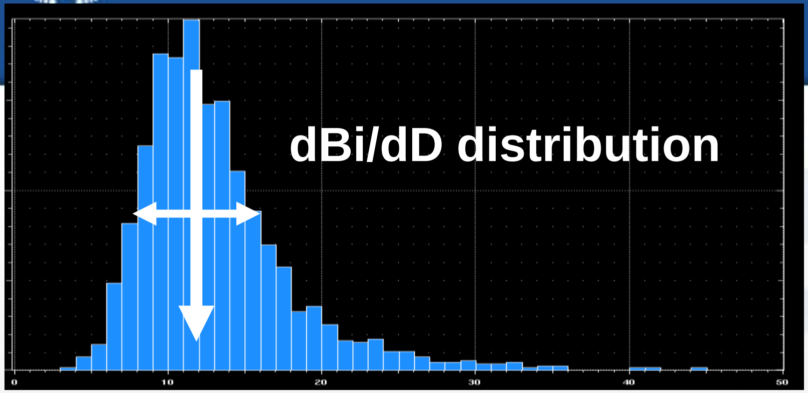


Non-specific Radiation Damage model:
Atomic Debye-Waller factor variation with Dose



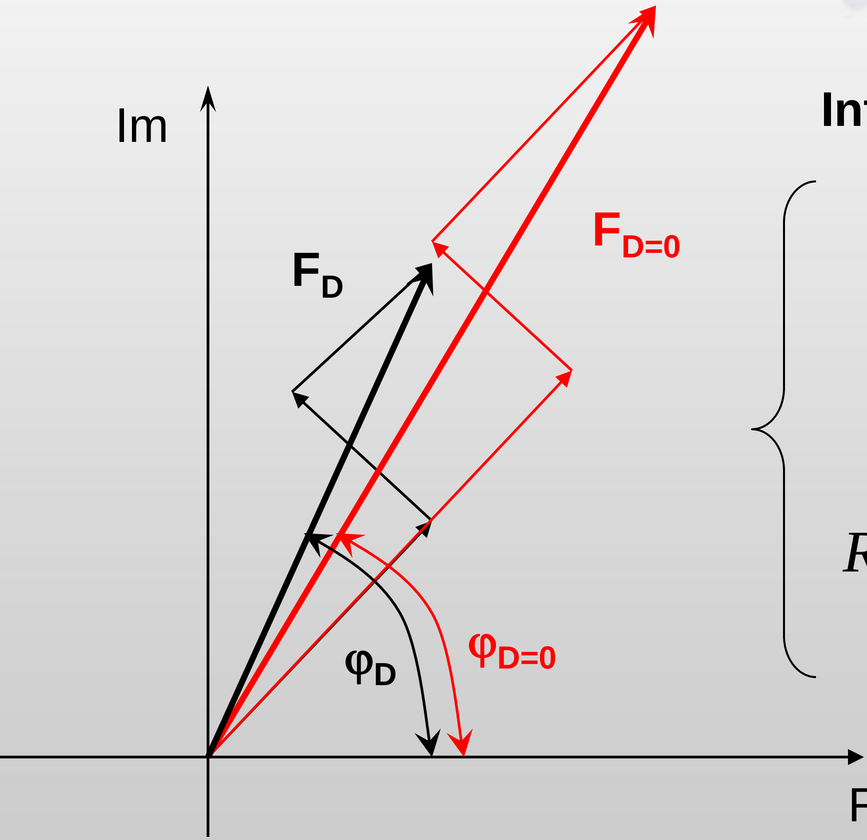


- Derived from the series of refined structures as a function of Dose @ constant I/SigI/resolution
- Not visible in electron density maps (other then blurring)
- Generates extreme non-isomorphism (>70% in R_{E^2})



Intensity decay:

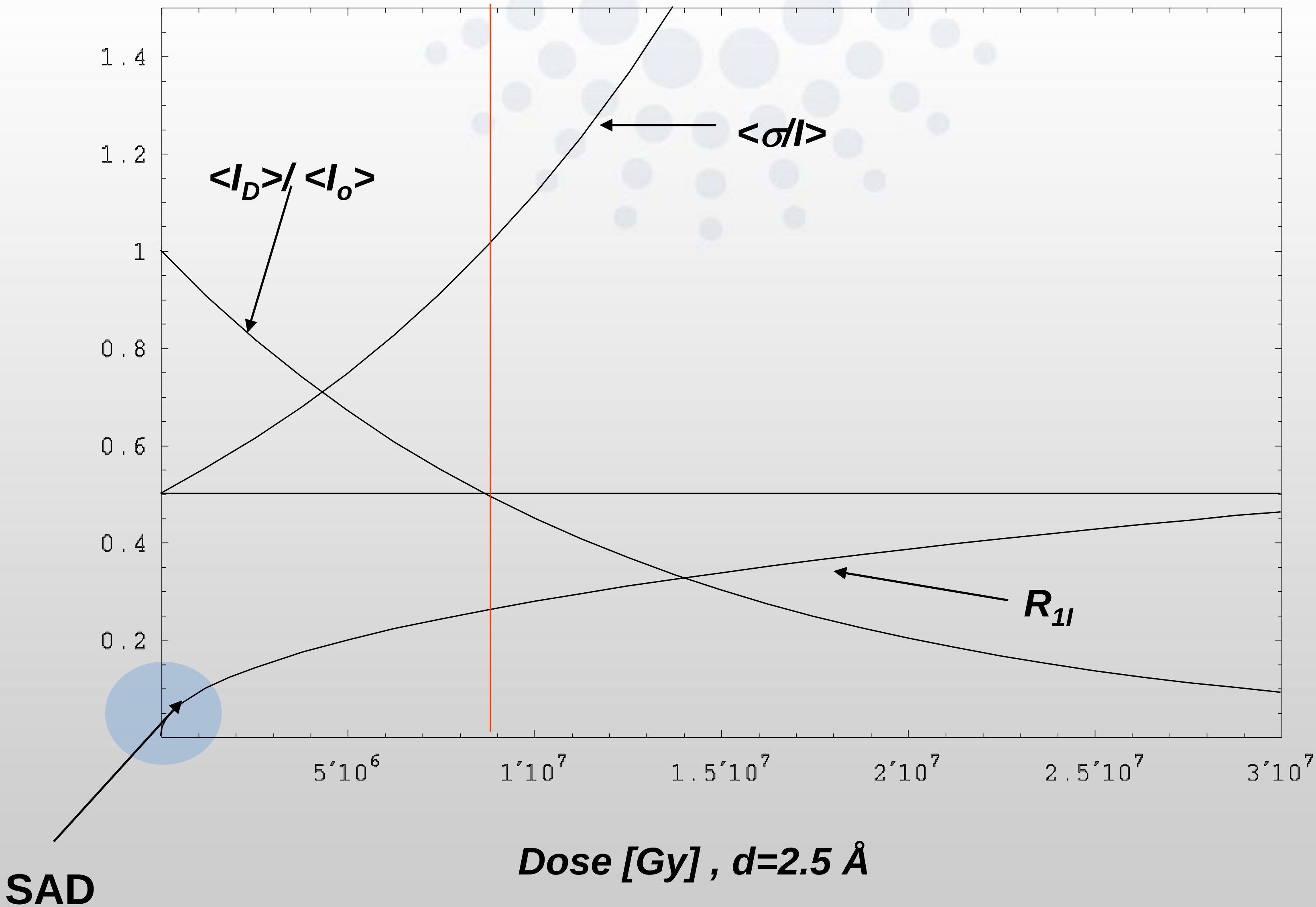
$$\frac{\langle I_D \rangle}{\langle I_{D=0} \rangle} \approx \left\langle e^{-2\frac{dBi}{dD}Ds^2} \right\rangle \approx e^{-2\beta Ds^2}$$

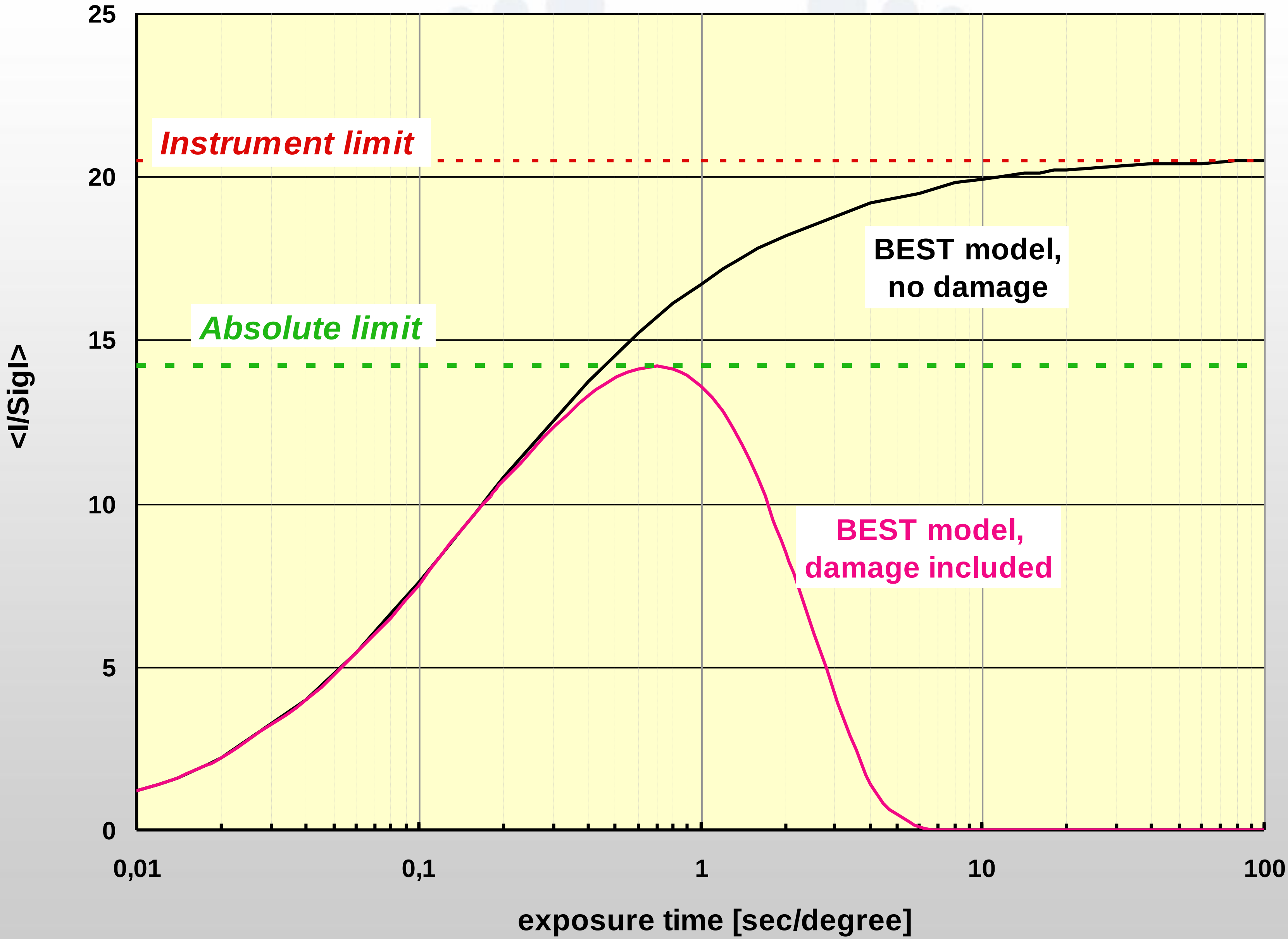


Intensity variance (non-isomorphism):

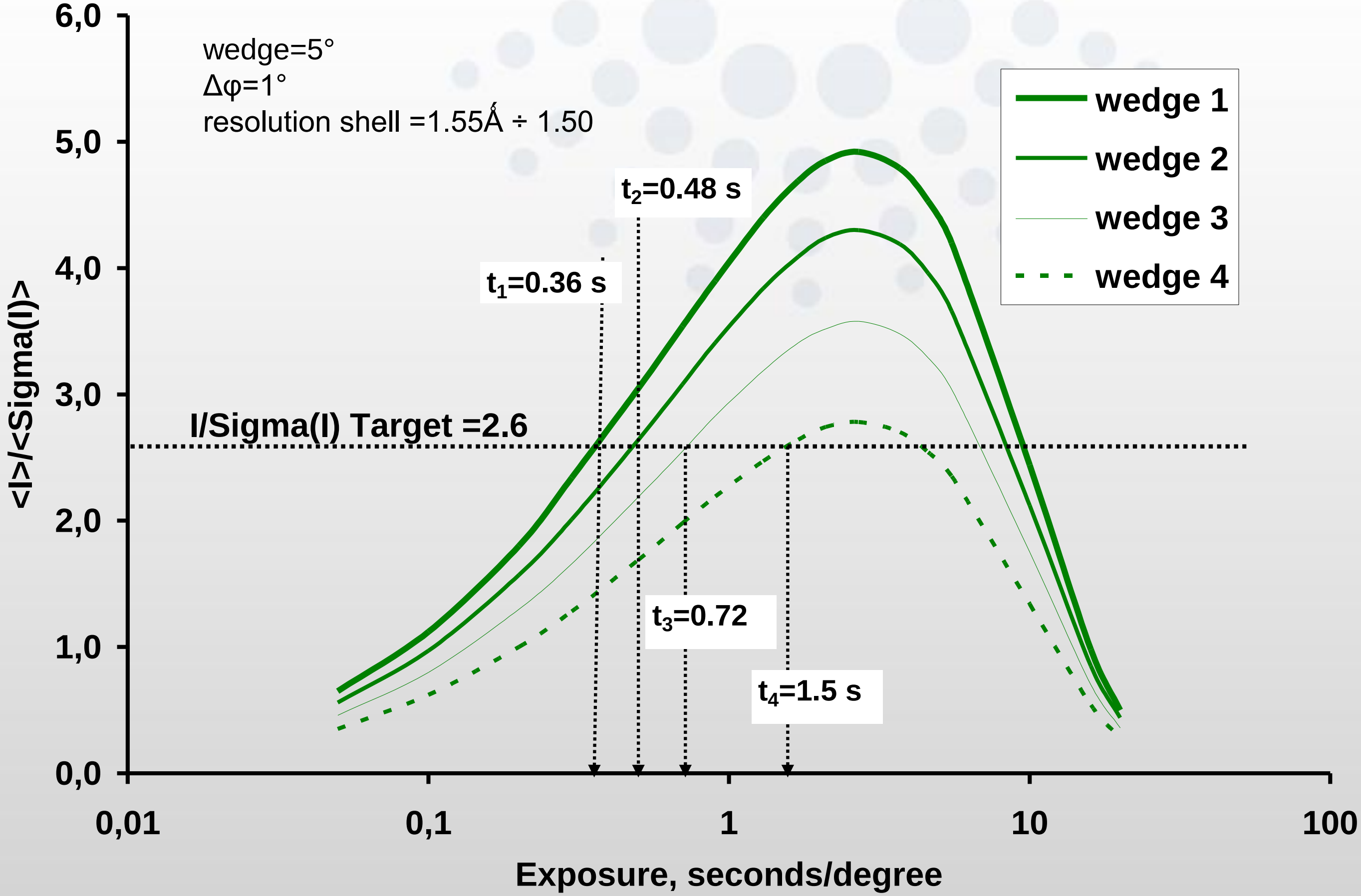
$$\begin{cases} \sigma_a \approx \left\langle e^{-\frac{dBi}{dD}Ds^2} \right\rangle \left\langle e^{-2\frac{dBi}{dD}Ds^2} \right\rangle^{-1/2} \approx e^{-\alpha Ds^2} \\ R_{1I} = \left\langle \left| \frac{I_{D=0}}{\langle I_{D=0} \rangle} - \frac{I_D}{\langle I_D \rangle} \right| \right\rangle \approx (1-\sigma_a^2)^{1/2} \end{cases}$$

Expected Intensity Variation




Instrument limit
Absolute limit
BEST model, no damage
BEST model, damage included
<I/SigI>
25
20
15
10
5
0
0,01
0,1
1
10
100
exposure time [sec/degree]

Insulin data collection



Graphical solution of the equation at resolution 1.5 Å and fixed rot. width for 5° sequential rotation wedges.

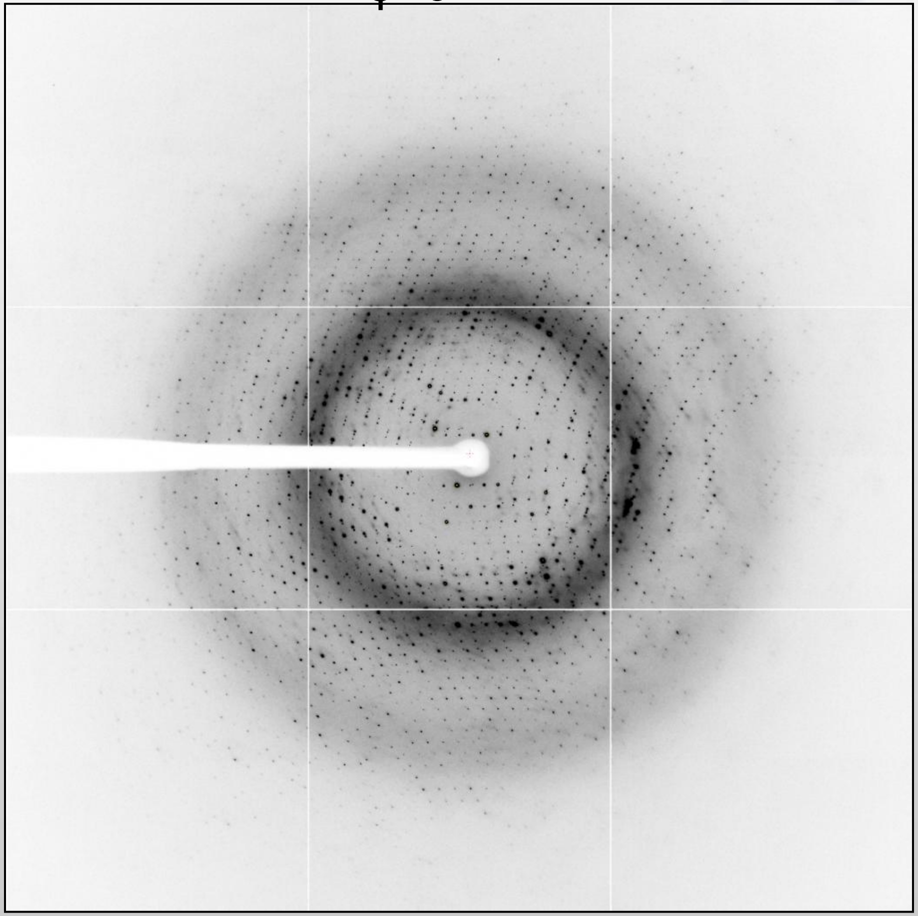
$$\frac{\hat{J}}{\hat{\sigma}_J}(t_{exposure}, D) = \frac{C}{\sqrt{M}} = \frac{4.0}{\sqrt{2.4}} = 2.58$$

ALCOHOL DEHYDROGENASE

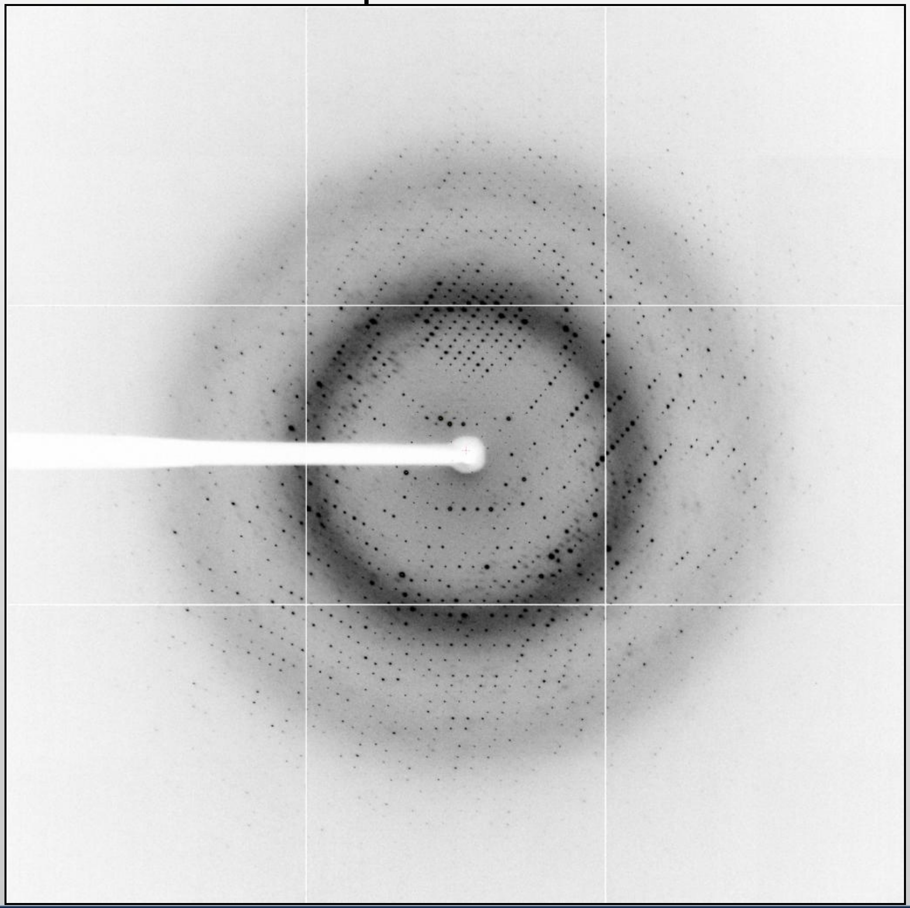
ID23-1, λ=0.973 Å, Flux=2.5 10^{12}
resolution =1.45 Å, Δφ=1º, t exposure=0.2 s,

Space Group : C 1 2 1
Cell : 148.7 53.6 76.5 90.0 103.6 90.0
Mosaicity : 0.40 degree

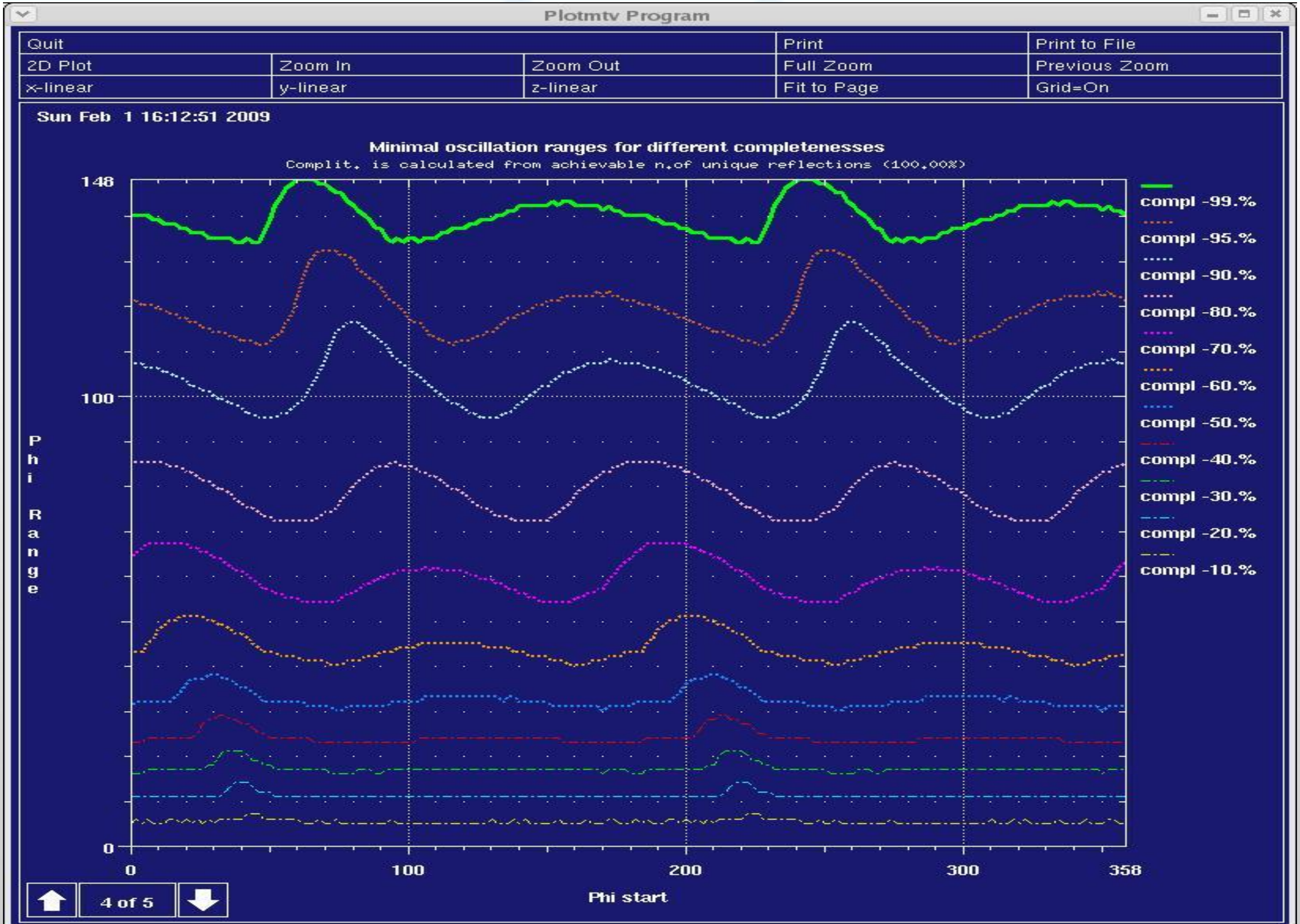
φ=0º

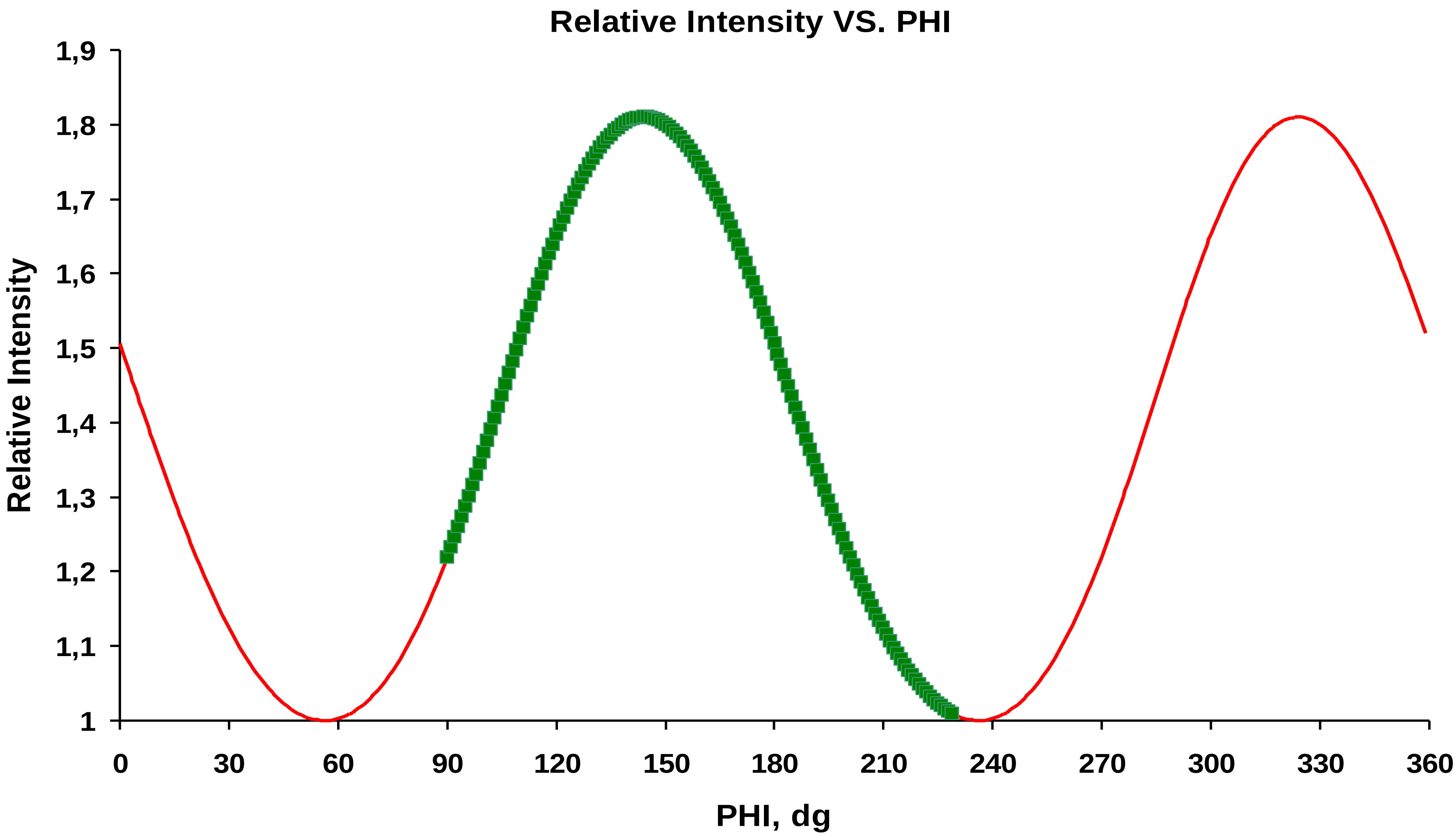


φ=90º

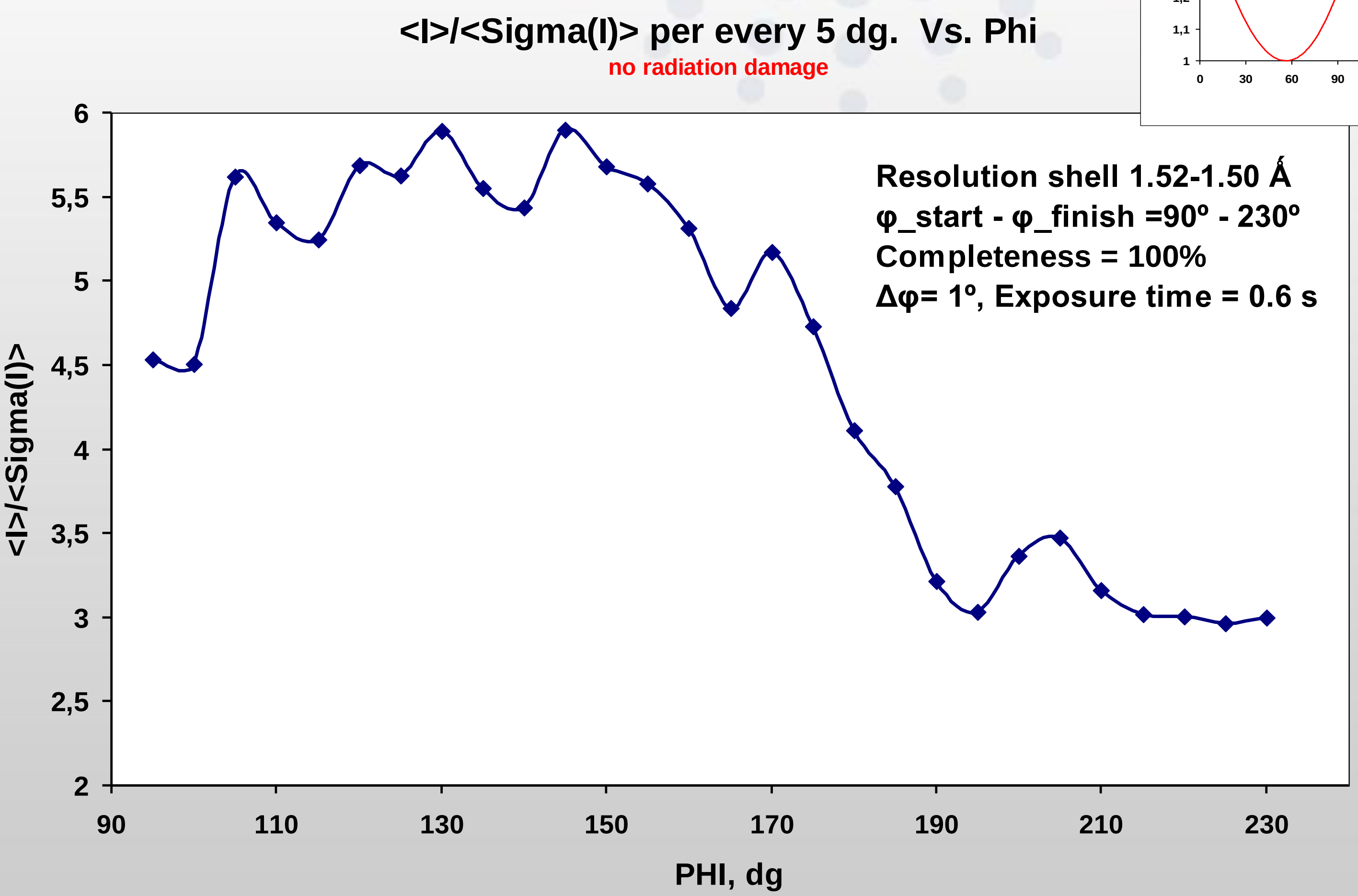


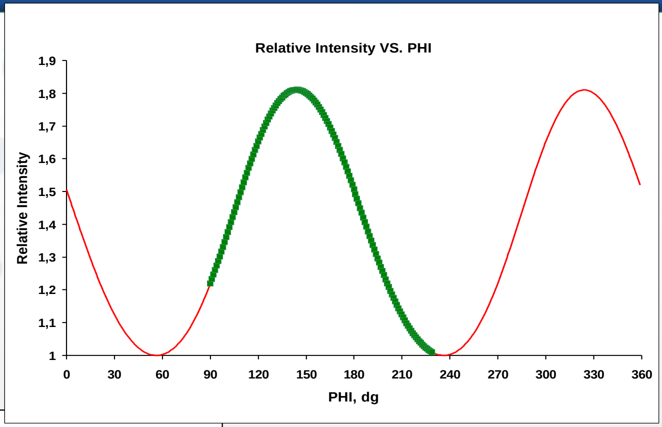
ALCOHOL DEHYDROGENASE

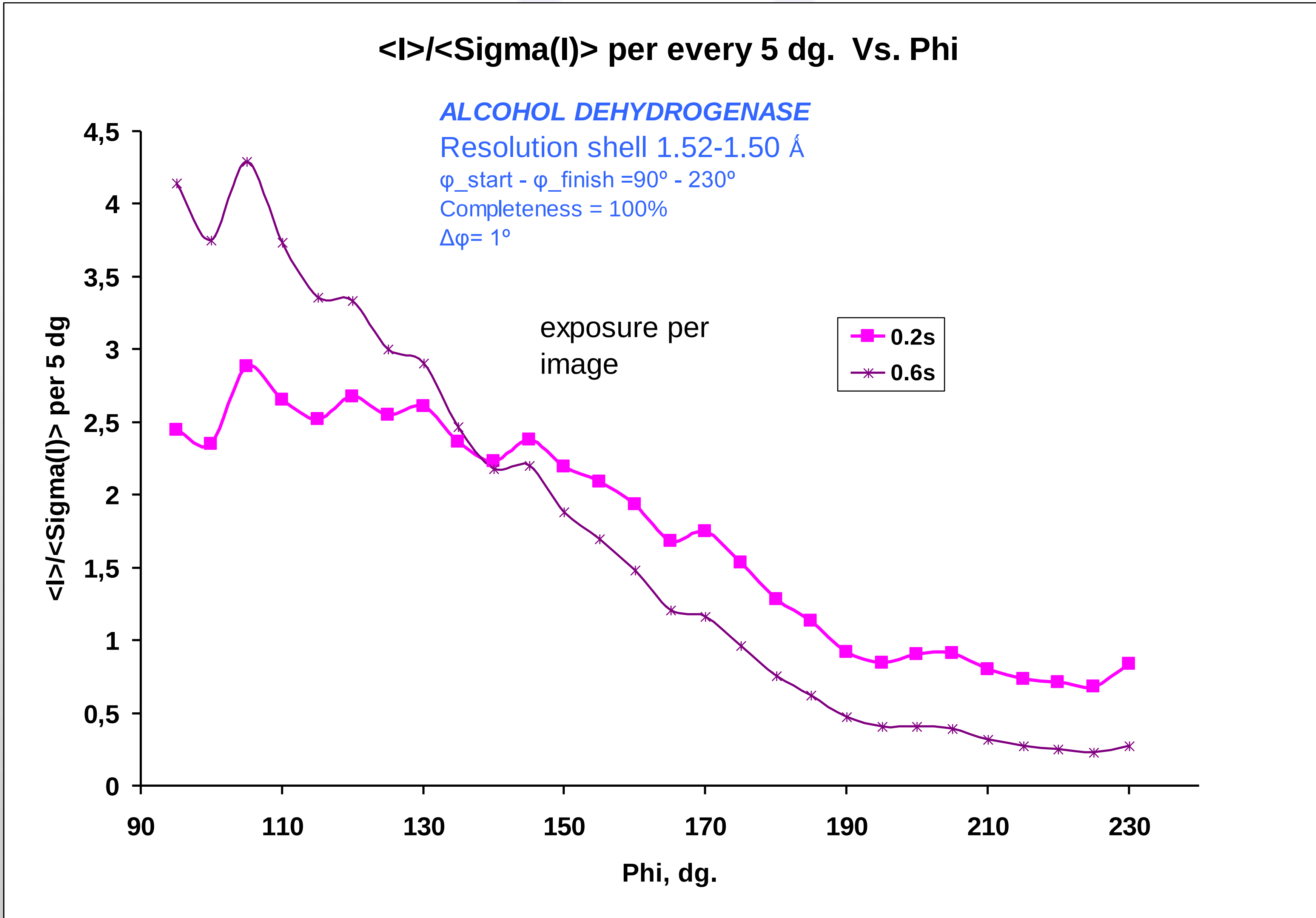


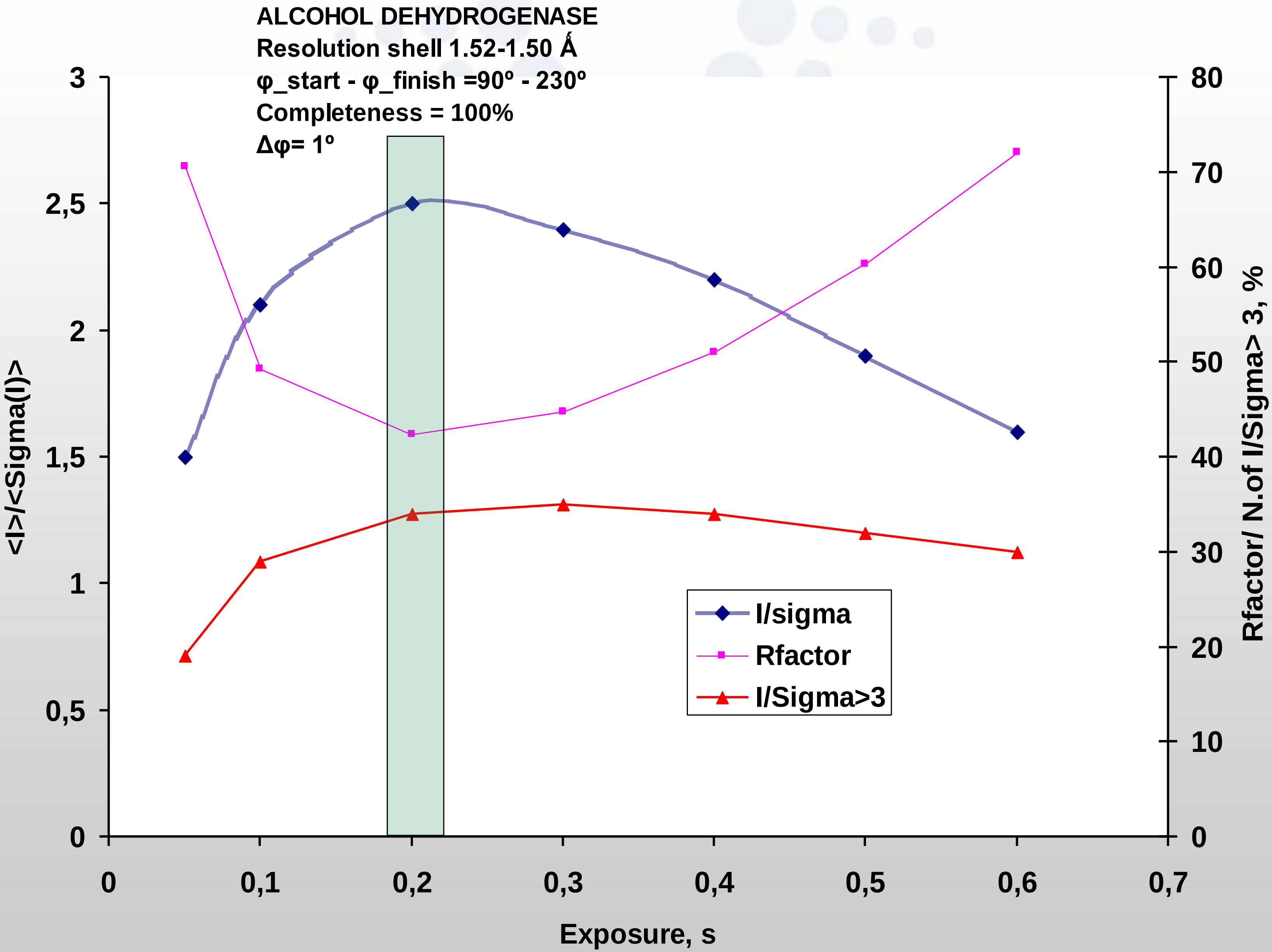

Relative Intensity VS. PHI
Relative Intensity
1,9
1,8
1,7
1,6
1,5
1,4
1,3
1,2
1,1
1
0
30
60
90
120
150
180
210
240
270
300
330
360
PHI, dg

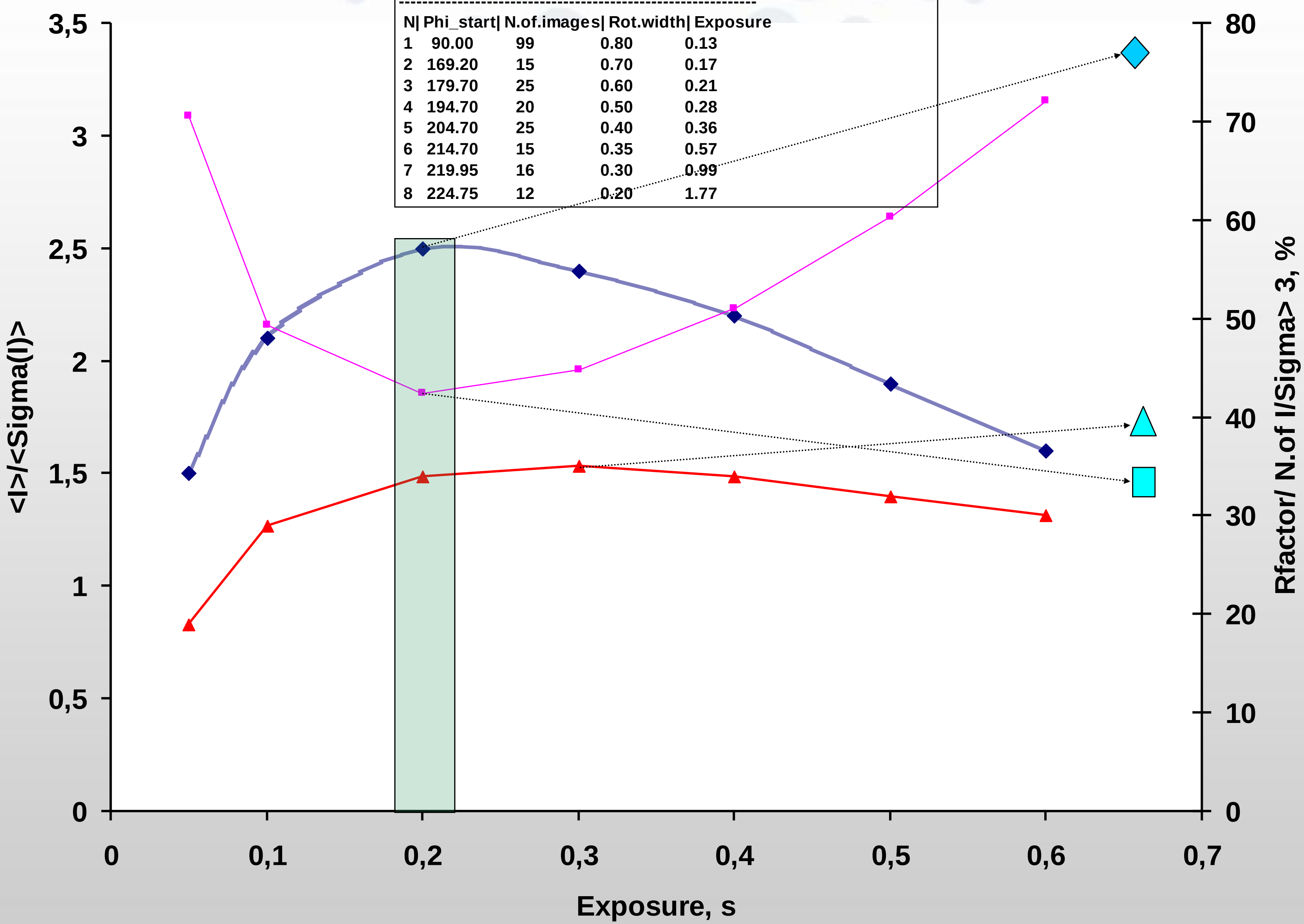
ALCOHOL DEHYDROGENASE






<I>/<Sigma(I)> per every 5 dg. Vs. Phi
ALCOHOL DEHYDROGENASE
Resolution shell 1.52-1.50 Ǻ
φ_start - φ_finish =90º - 230º
Completeness = 100%
Δφ= 1º
exposure per image
0.2s
0.6s
<I>/<Sigma(I)> per 5 dg
0
0,5
1
1,5
2
2,5
3
3,5
4
4,5
90
110
130
150
170
190
210
230
Phi, dg.


ALCOHOL DEHYDROGENASE
Resolution shell 1.52-1.50 Å
φ_start - φ_finish =90º - 230º
Completeness = 100%
Δφ= 1º
<I>/<Sigma(I)>
Rfactor/ N.of I/Sigma> 3, %
Exposure, s
I/sigma
Rfactor
I/Sigma>3
0
0,5
1
1,5
2
2,5
3
0
10
20
30
40
50
60
70
80
0
0,1
0,2
0,3
0,4
0,5
0,6
0,7



BEST Optimal Plan of data collection

N	Phi_start	N.of.images	Rot.width	Exposure
1	90.00	99	0.80	0.13
2	169.20	15	0.70	0.17
3	179.70	25	0.60	0.21
4	194.70	20	0.50	0.28
5	204.70	25	0.40	0.36
6	214.70	15	0.35	0.57
7	219.95	16	0.30	0.99
8	224.75	12	0.20	1.77

BEST

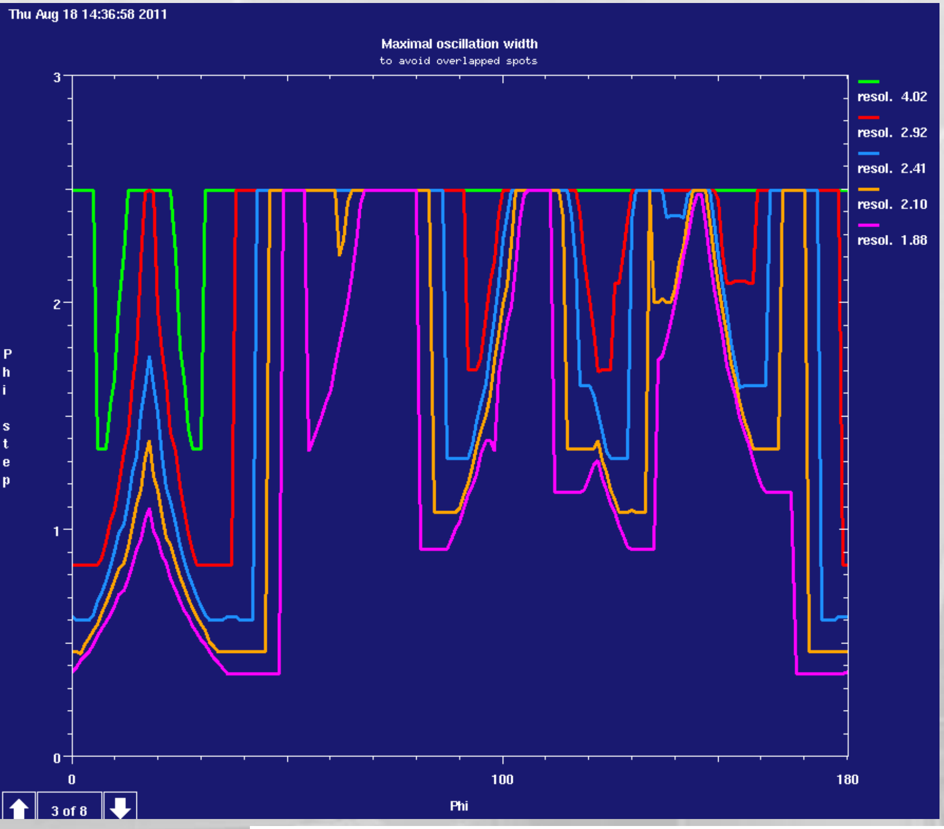
A light for Science

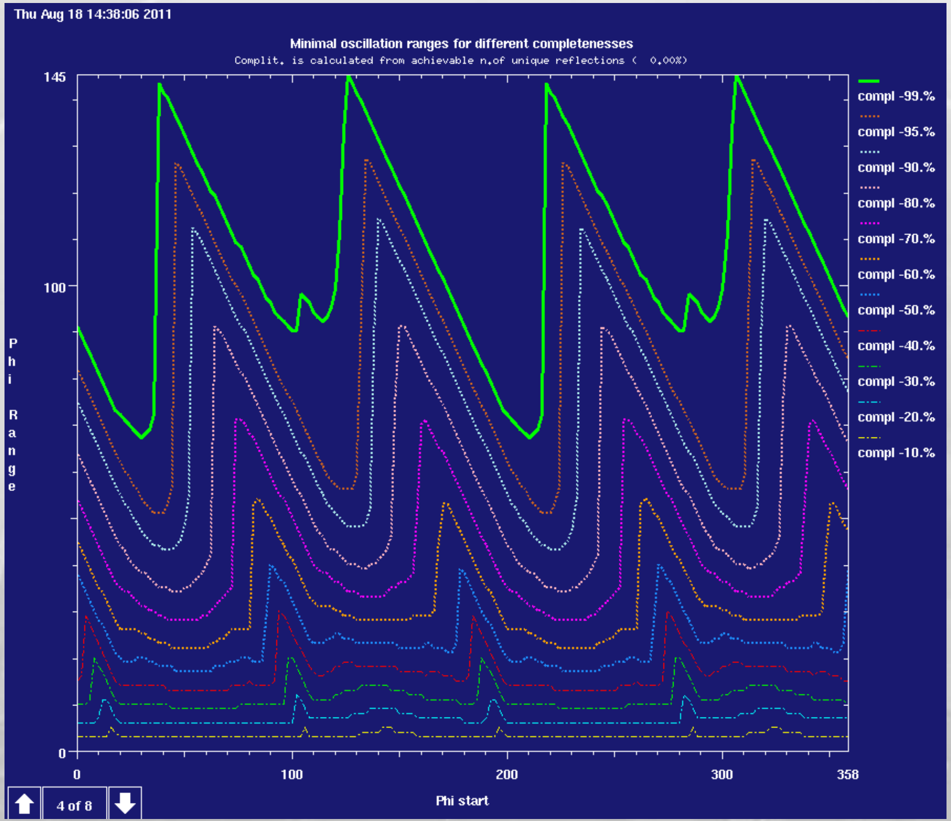
Geometry

Optimal starting spindle angle and scan range

Maximum rotation angle without spot overlap

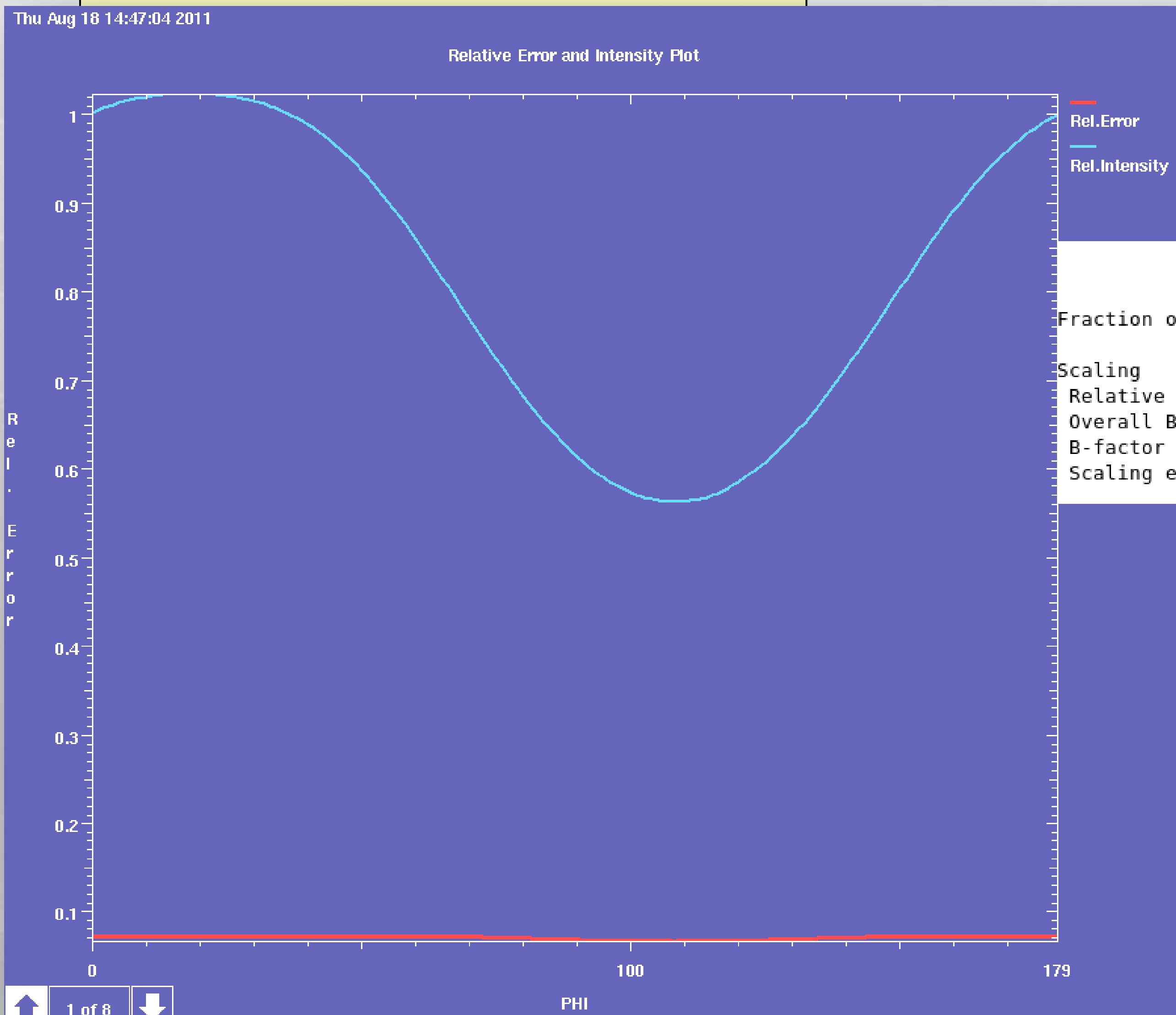
Optimal Multiplicity






```
Crystal
 Space Group                         : P 2 2 2
 Cell                                :  53.52  75.64 123.21  90.00  90.00  90.00
 Mosaicity                           : 0.51 degree
```

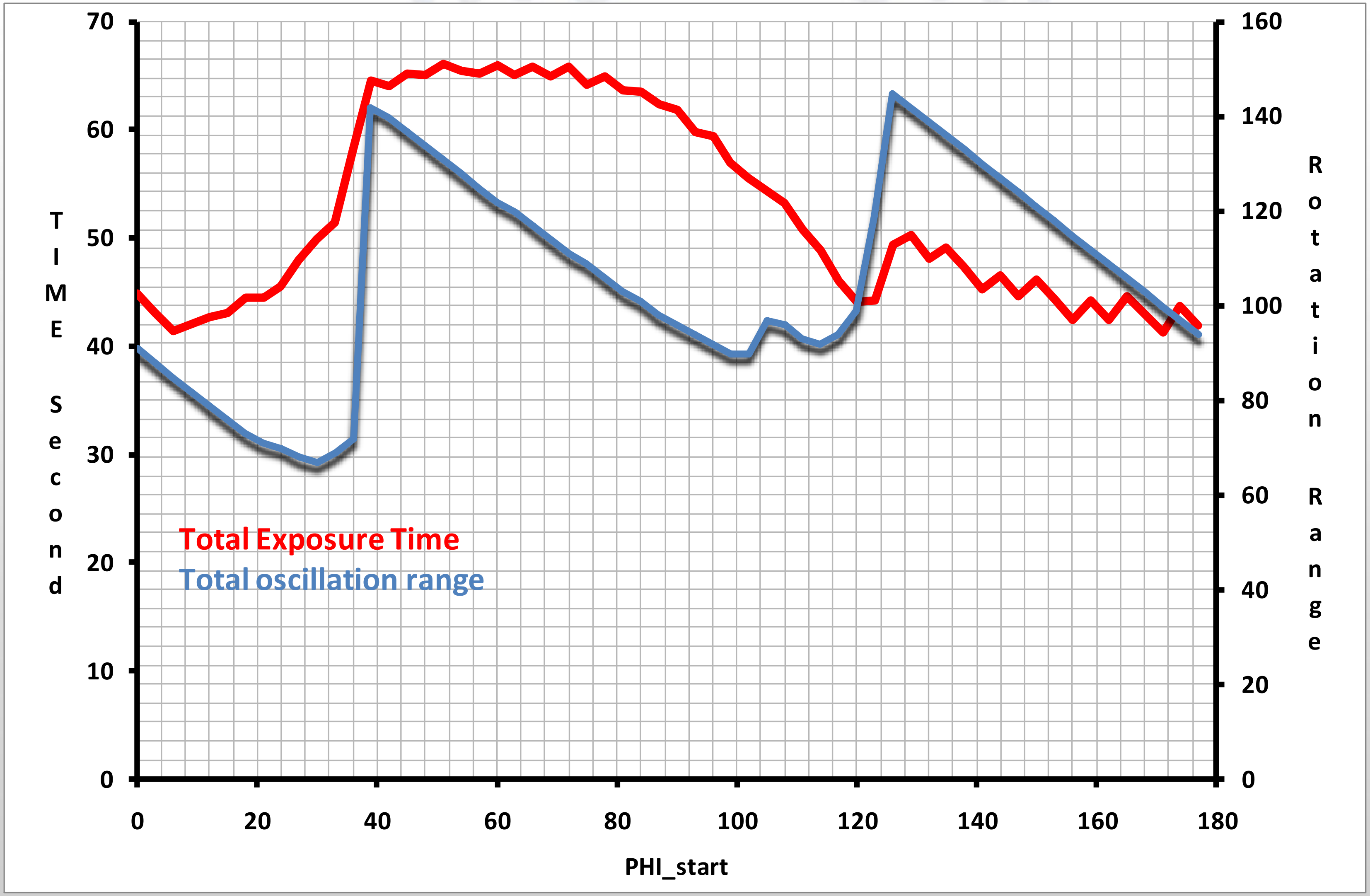
Intensity vs. crystal position




```
                Additional information
              ======================
Fraction of unique reflections in blind region:   2.7%

Scaling
 Relative scale        :    6.37
 Overall B-factor      :  36.48 Angstrom^2
 B-factor eigenvalues  :  31.86   35.88   43.51 Angstrom^2
 Scaling error         :   7% at the resolution limit
```

Optimal Oscillation Range



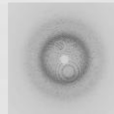
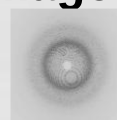
Data collection strategy accounting radiation damage

Beamline Flux
Crystal contents
Crystal sizes

RADDOSE

Absorbed dose rate

Initial Images

MOSFLM XDS DENZO

Space group
Cell parameters
Orientation
Mosaicity
I[(h,k,l))]
$I_{background}(h)$

User choices

- Optimize data collection
- Optimize SAD data collection
- Find optimal crystal orientation
- Low-resolution optimal
- Rad. Damage sensitivity
- Multi-positional data collection
- Estimate data statistics

Detector parameters
Beamline parameters
and limitations

BEST 3.4.3

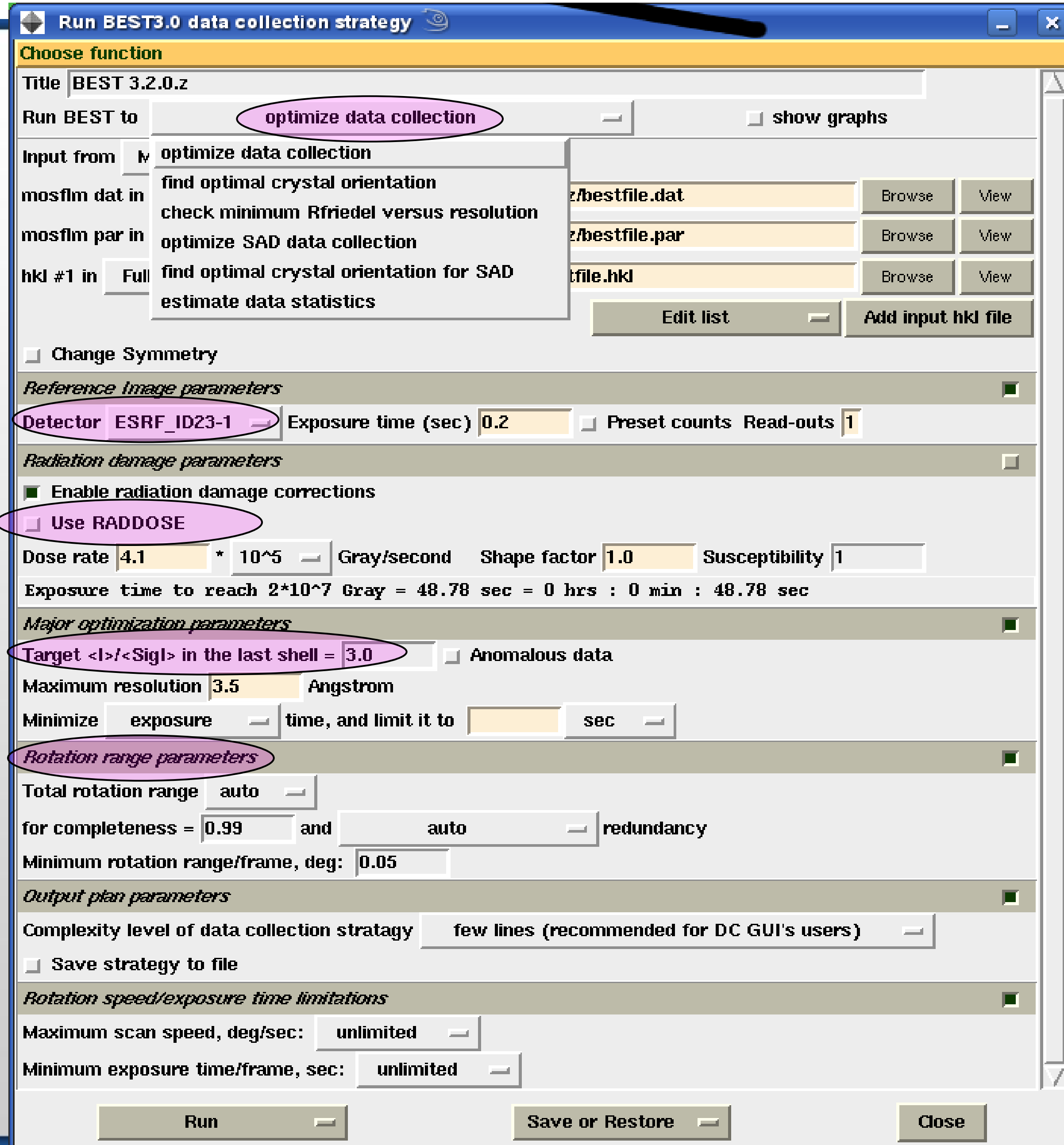
Dose (Time) limit
Geometry limits
Aimed statistics
Aimed completeness
Aimed redundancy
Aimed resolution

Optimal plan(s) of data collection
Resolution limit
Statistics
B-factor

BEST
http://www.embl-hamburg.de/BEST/

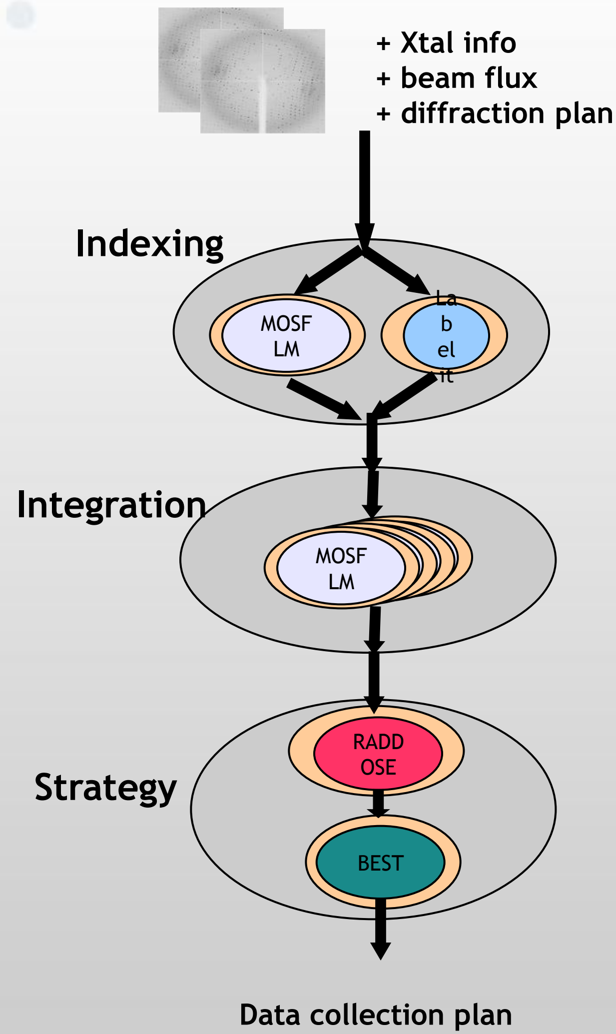
Popov & Bourenkov, Acta Crystallogr. (2003). D59, 1145-1153
Bourenkov & Popov, Acta Crystallogr. (2006). D62, 58-64
Bourenkov & Popov, Acta Crystallogr. (2010). D66, 409-419

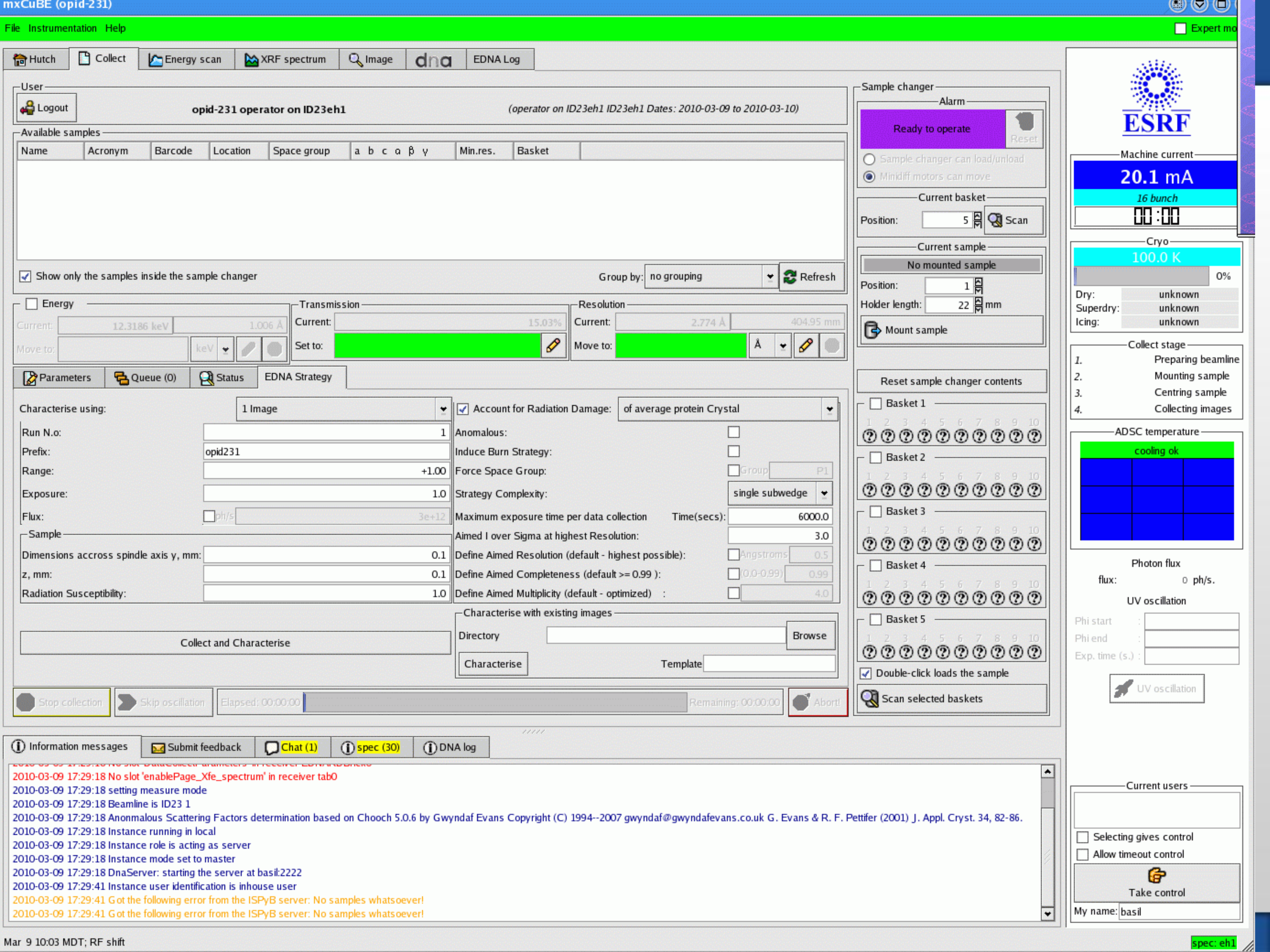
Interface:CCP4I BEST

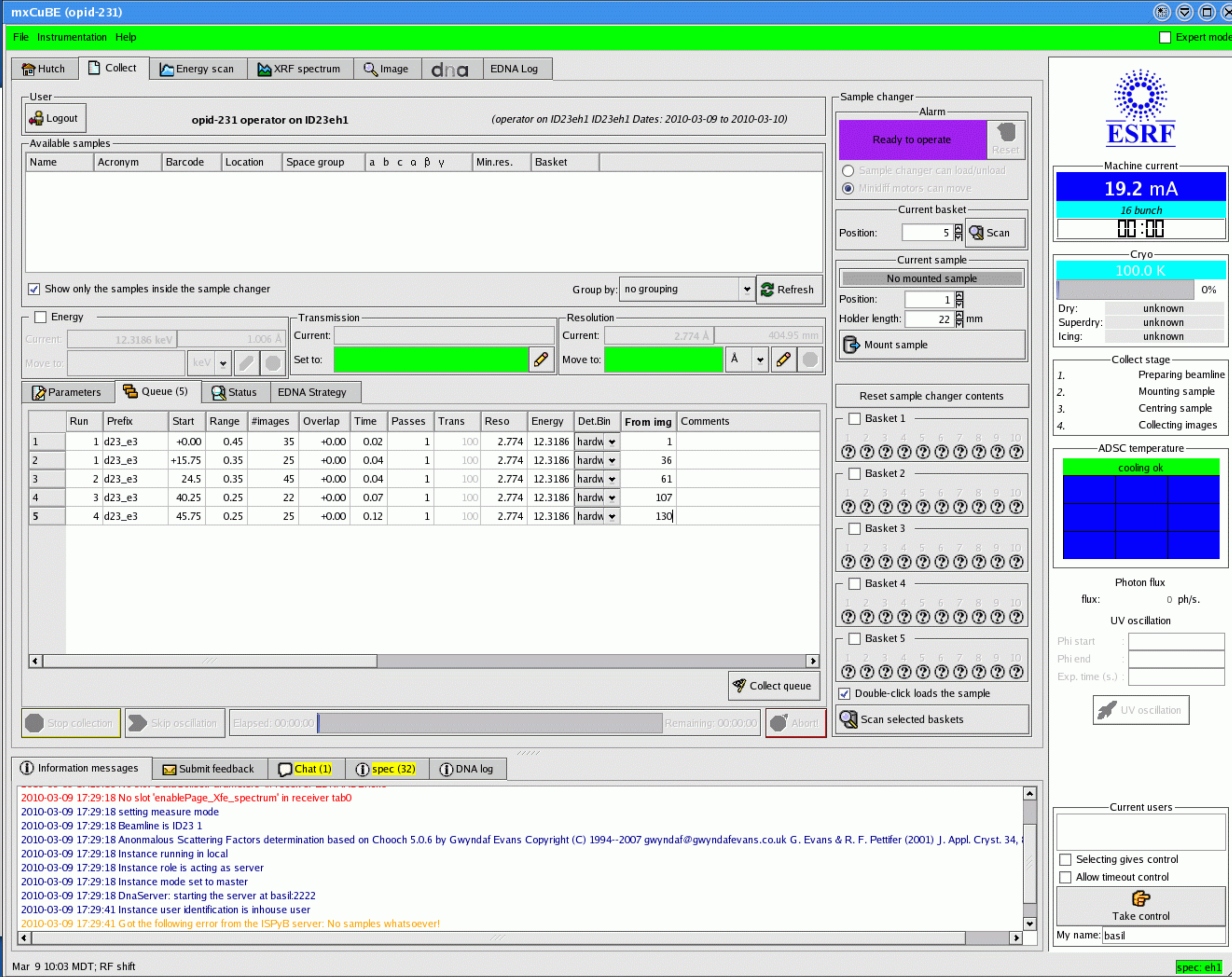


EDNA MXv1 Characterisation

- MX sample characterisation taking into account radiation damage
- Indexing using MOSFLM or Labelit
- Parallel integration of reference images
- If flux + beamsize + chemical composition:
  - RADDOSE for estimating dose rate (Gy/s)
- BEST strategy calculation
  - taking into account radiation damage
  - multi-subwedge data collection strategies




mxCuBE (opid-231)
File Instrumentation Help
Expert mo
Hutch
Collect
Energy scan
XRF spectrum
Image
dna
EDNA Log
User
Logout
opid-231 operator on ID23eh1
(operator on ID23eh1 ID23eh1 Dates: 2010-03-09 to 2010-03-10)
Available samples
Name
Acronym
Barcode
Location
Space group
a b c α β γ
Min.res.
Basket
Show only the samples inside the sample changer
Group by:
no grouping
Refresh
Energy
Current:
12.3186 keV
1.006 Å
Move to:
keV
Transmission
Current:
15.03%
Set to:
Resolution
Current:
2.774 Å
404.95 mm
Move to:
Å
Parameters
Queue (0)
Status
EDNA Strategy
Characterise using:
1 Image
Run N.o:
1
Prefix:
opid231
Range:
+1.00
Exposure:
1.0
Flux:
ph/s
3e+12
Sample
Dimensions accross spindle axis y, mm:
0.1
z, mm:
0.1
Radiation Susceptibility:
1.0
Collect and Characterise
Account for Radiation Damage:
of average protein Crystal
Anomalous:
Induce Burn Strategy:
Force Space Group:
Group
P1
Strategy Complexity:
single subwedge
Maximum exposure time per data collection
Time(secs):
6000.0
Aimed I over Sigma at highest Resolution:
3.0
Define Aimed Resolution (default - highest possible):
Angstroms
0.5
Define Aimed Completeness (default >= 0.99):
(0.0-0.99)
0.99
Define Aimed Multiplicity (default - optimized) :
4.0
Characterise with existing images
Directory
Browse
Characterise
Template
Stop collection
Skip oscillation
Elapsed: 00:00:00
Remaining: 00:00:00
Abort!
Sample changer
Alarm
Ready to operate
Reset
Sample changer can load/unload
Minidiff motors can move
Current basket
Position:
5
Scan
Current sample
No mounted sample
Position:
1
Holder length:
22
mm
Mount sample
Reset sample changer contents
Basket 1
Basket 2
Basket 3
Basket 4
Basket 5
1 2 3 4 5 6 7 8 9 10
Double-click loads the sample
Scan selected baskets
ESRF
Machine current
20.1 mA
16 bunch
00:00
Cryo
100.0 K
0%
Dry:
unknown
Superdry:
unknown
Icing:
unknown
Collect stage
1. Preparing beamline
2. Mounting sample
3. Centring sample
4. Collecting images
ADSC temperature
cooling ok
Photon flux
flux: 0 ph/s.
UV oscillation
Phi start
Phi end
Exp. time (s.)
UV oscillation
Current users
Selecting gives control
Allow timeout control
Take control
My name: basil
Information messages
Submit feedback
Chat (1)
spec (30)
DNA log
2010-03-09 17:29:18 No slot 'enablePage_Xfe_spectrum' in receiver tab0
2010-03-09 17:29:18 setting measure mode
2010-03-09 17:29:18 Beamline is ID23 1
2010-03-09 17:29:18 Anonmalous Scattering Factors determination based on Chooch 5.0.6 by Gwyndaf Evans Copyright (C) 1994--2007 gwyndaf@gwyndafevans.co.uk G. Evans & R. F. Pettifer (2001) J. Appl. Cryst. 34, 82-86.
2010-03-09 17:29:18 Instance running in local
2010-03-09 17:29:18 Instance role is acting as server
2010-03-09 17:29:18 Instance mode set to master
2010-03-09 17:29:18 DnaServer: starting the server at basil:2222
2010-03-09 17:29:41 Instance user identification is inhouse user
2010-03-09 17:29:41 Got the following error from the ISPyB server: No samples whatsoever!
2010-03-09 17:29:41 Got the following error from the ISPyB server: No samples whatsoever!
Mar 9 10:03 MDT; RF shift
spec: eh1



mxCuBE (opid-231)
File Instrumentation Help
Expert mode
Hutch
Collect
Energy scan
XRF spectrum
Image
dna
EDNA Log
User
Logout
opid-231 operator on ID23eh1
(operator on ID23eh1 ID23eh1 Dates: 2010-03-09 to 2010-03-10)
Available samples
Name Acronym Barcode Location Space group a b c α β γ Min.res. Basket
Show only the samples inside the sample changer
Group by: no grouping
Refresh
Energy
Current: 12.3186 keV 1.006 Å
Move to: keV
Transmission
Current:
Set to:
Resolution
Current: 2.774 Å 404.95 mm
Move to: Å
Parameters
Queue (5)
Status
EDNA Strategy
Run Prefix Start Range #images Overlap Time Passes Trans Reso Energy Det.Bin From img Comments
1 1 d23_e3 +0.00 0.45 35 +0.00 0.02 1 100 2.774 12.3186 hardw 1
2 1 d23_e3 +15.75 0.35 25 +0.00 0.04 1 100 2.774 12.3186 hardw 36
3 2 d23_e3 24.5 0.35 45 +0.00 0.04 1 100 2.774 12.3186 hardw 61
4 3 d23_e3 40.25 0.25 22 +0.00 0.07 1 100 2.774 12.3186 hardw 107
5 4 d23_e3 45.75 0.25 25 +0.00 0.12 1 100 2.774 12.3186 hardw 130
Collect queue
Stop collection
Skip oscillation
Elapsed: 00:00:00
Remaining: 00:00:00
Abort!
Sample changer
Alarm
Ready to operate
Reset
Sample changer can load/unload
Minidiff motors can move
Current basket
Position: 5
Scan
Current sample
No mounted sample
Position: 1
Holder length: 22 mm
Mount sample
Reset sample changer contents
Basket 1
Basket 2
Basket 3
Basket 4
Basket 5
Double-click loads the sample
Scan selected baskets
ESRF
Machine current
19.2 mA
16 bunch
Cryo
100.0 K
0%
Dry: unknown
Superdry: unknown
Icing: unknown
Collect stage
1. Preparing beamline
2. Mounting sample
3. Centring sample
4. Collecting images
ADSC temperature
cooling ok
Photon flux
flux: 0 ph/s.
UV oscillation
Phi start
Phi end
Exp. time (s.)
UV oscillation
Information messages
Submit feedback
Chat (1)
spec (32)
DNA log
2010-03-09 17:29:18 No slot 'enablePage_Xfe_spectrum' in receiver tab0
2010-03-09 17:29:18 setting measure mode
2010-03-09 17:29:18 Beamline is ID23 1
2010-03-09 17:29:18 Anonmalous Scattering Factors determination based on Chooch 5.0.6 by Gwyndaf Evans Copyright (C) 1994--2007 gwyndaf@gwyndafevans.co.uk G. Evans & R. F. Pettifer (2001) J. Appl. Cryst. 34,
2010-03-09 17:29:18 Instance running in local
2010-03-09 17:29:18 Instance role is acting as server
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2010-03-09 17:29:18 DnaServer: starting the server at basil:2222
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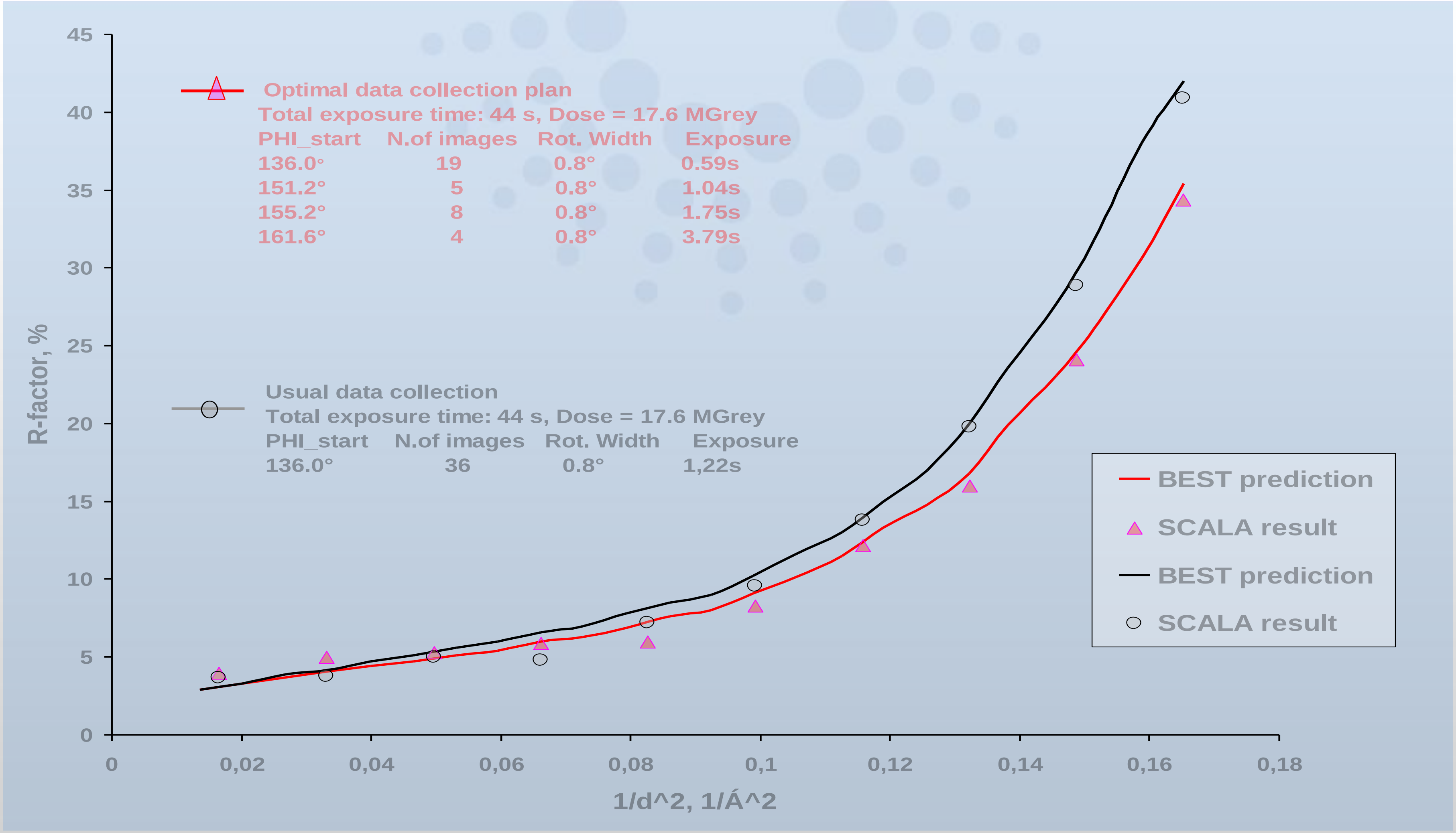
Current users
Selecting gives control
Allow timeout control
Take control
My name: basil
Mar 9 10:03 MDT; RF shift
spec: eh1



Test data collection of a complex of viral supressor of RNA crystal according to the BEST plan.Two full data sets were measured at resolution 2.45 Å with equal dose using two different crystal parts from long (~ 0.2 mm) and thin (~0.025 mm) sample on ID23-1

Good native data ⇔ Poor anomalous data

Data collection statistics according to the plan

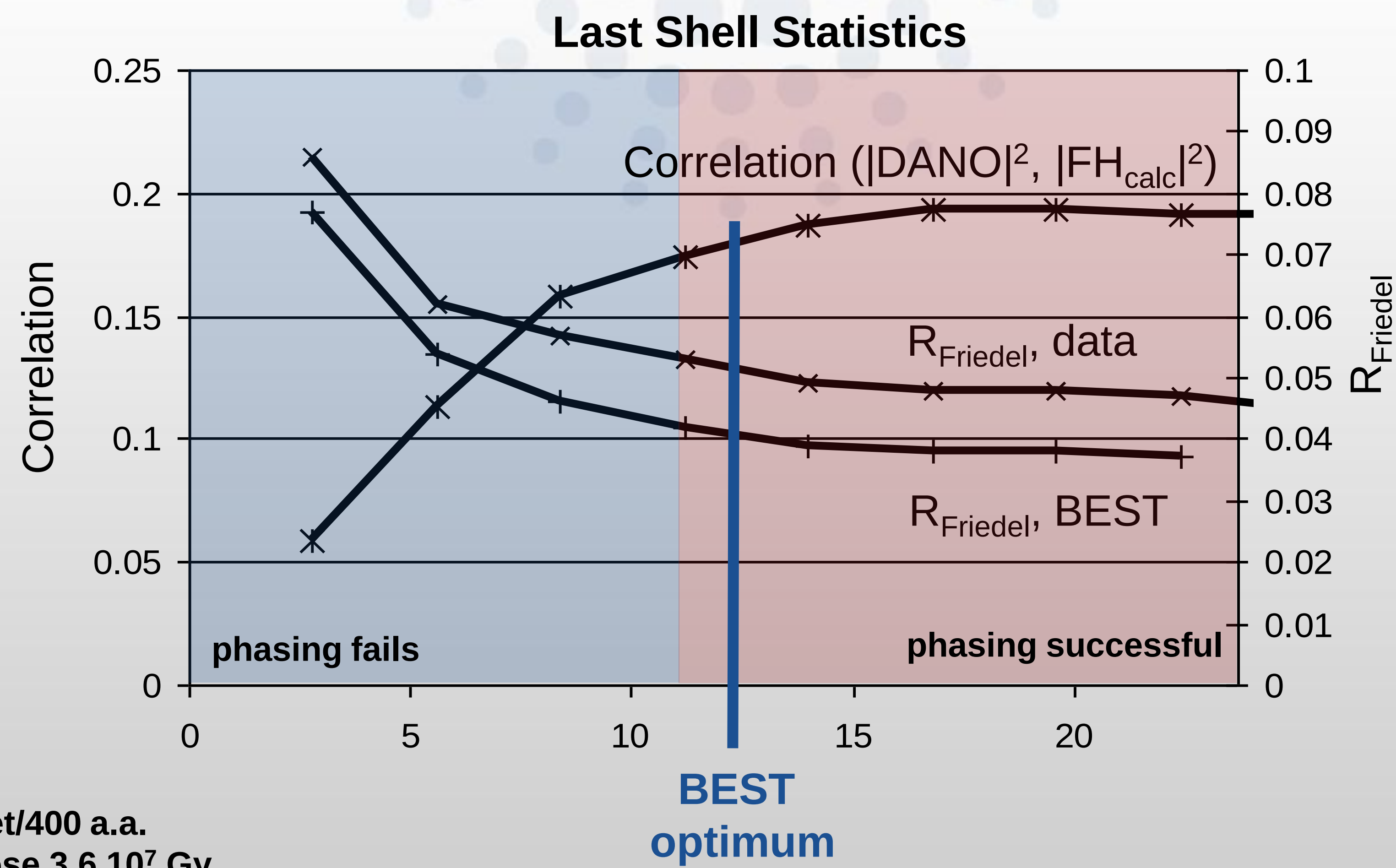
Resolution Lower	Resolution Upper	Compl. %	Average Intensity	Average Sigma	I/Sigma stat	I/Sigma /Chi	Chi**2	R-fact %	Ranom %	Overload %
12.00	7.15	94.5	16009.6	611.2	26.2	25.5	1.06	3.8	3.7	0.02
7.15	5.57	97.6	8635.2	342.5	25.2	23.9	1.11	4.0	4.7	0.00
5.57	4.72	97.2	11871.6	471.7	25.2	22.9	1.20	4.2	5.3	0.00
4.72	4.17	97.3	15338.3	612.9	25.0	22.0	1.30	4.4	5.9	0.00
4.17	3.78	97.6	12968.2	522.9	24.8	21.0	1.40	4.7	6.5	0.00
3.78	3.48	97.4	10315.0	418.7	24.6	19.8	1.54	4.9	6.9	0.00
3.48	3.24	98.0	7715.0	318.4	24.2	18.7	1.68	5.3	7.4	0.00
3.24	3.04	98.1	5434.8	231.0	23.5	17.5	1.82	5.6	8.0	0.00
3.04	2.88	98.9	3988.7	174.2	22.9	16.5	1.93	6.0	8.3	0.00
2.88	2.74	98.4	2988.1	138.4	21.6	15.0	2.07	6.6	8.8	0.00
2.74	2.62	98.9	2391.1	117.2	20.4	14.2	2.08	6.9	9.2	0.00
2.62	2.51	99.5	1989.6	104.8	19.0	12.9	2.15	7.5	9.8	0.00
2.51	2.42	99.5	1690.5	95.8	17.6	12.1	2.14	8.1	10.3	0.00
2.42	2.33	100.0	1487.5	90.1	16.5	11.3	2.12	8.7	10.7	0.00
2.33	2.26	100.0	1308.6	86.9	15.1	10.4	2.09	9.3	11.1	0.00
2.26	2.19	100.0	1157.2	84.2	13.7	9.7	2.01	10.0	11.8	0.00
2.19	2.12	100.0	982.7	81.5	12.1	8.8	1.86	10.9	12.2	0.00
2.12	2.07	100.0	843.6	79.3	10.6	8.0	1.77	11.9	13.0	0.00
2.07	2.01	100.0	687.1	77.8	8.8	7.0	1.60	13.6	14.1	0.00
2.01	1.96	100.0	550.9	76.0	7.2	6.1	1.43	15.5	15.4	0.00
1.96	1.92	100.0	433.0	74.1	5.8	5.1	1.32	18.5	17.5	0.00
1.92	1.87	100.0	340.3	75.4	4.5	4.1	1.20	22.1	20.4	0.00
All data		99.2	3437.7	169.0	20.3	15.5	1.73	5.9	7.6	0.00

SAD optimization

Minimum of $R_{Friedel} = \langle|\langle E^{2+}\rangle - \langle E^{2-}\rangle|\rangle$ is a target

noise only, no anomalous scattering itself:
decay, non-isomorphism
exact pair-vice dose differences for Bijvoet mates

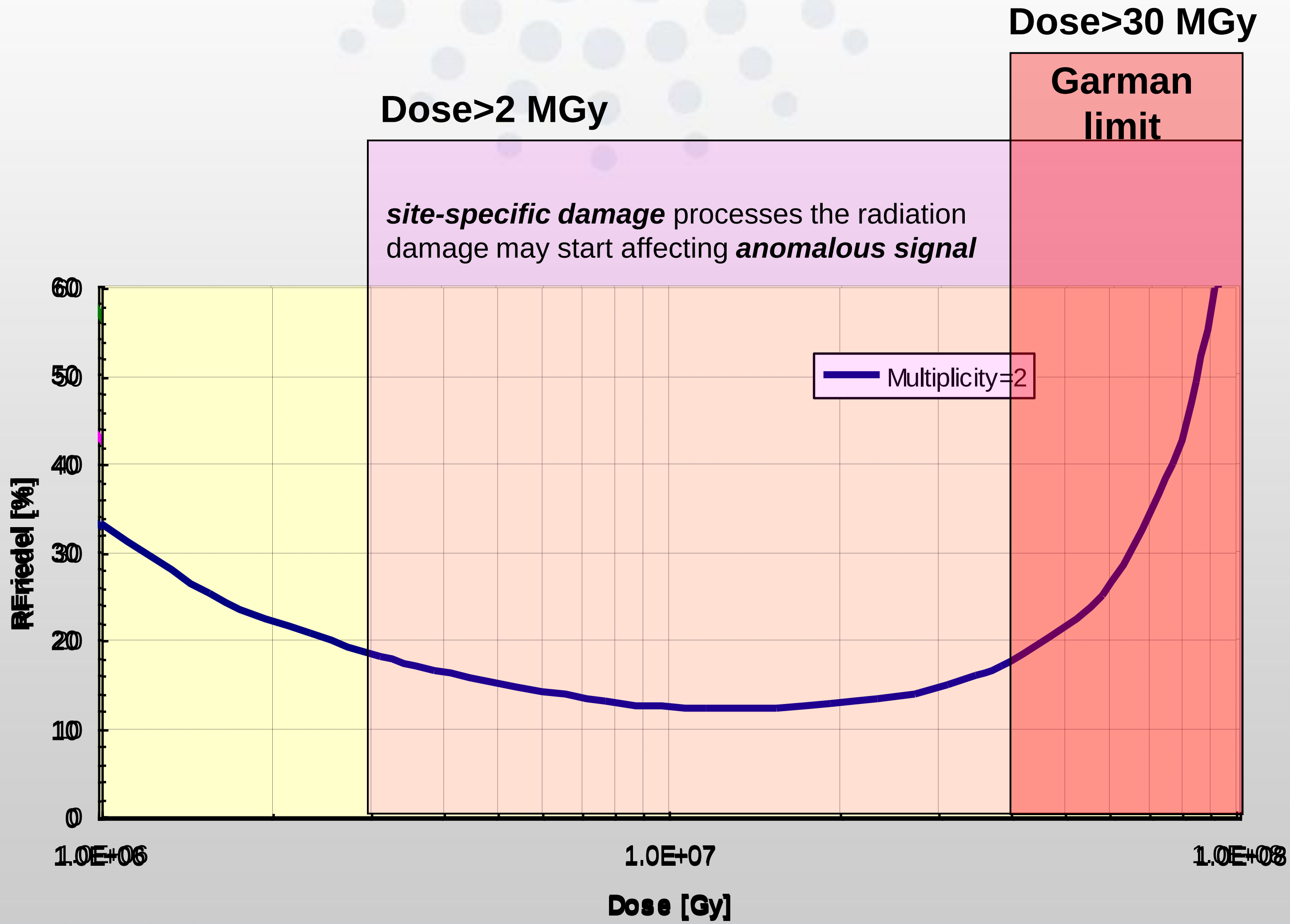
Testing: multiplicity dependency



3.3 Å
11 SeMet/400 a.a.
Total Dose 3.6 10^7 Gy
Constant rotation

SAD optimization

Minimum of $R_{Friedel} = \langle|\langle E^{2+}\rangle - \langle E^{2-}\rangle|\rangle$ is a target



SAD optimization

Minimum of RFriedel = <|<E2+/w>-<E2-/w>|> is a target
noise only, no anomalous scattering itself:
decay, non-isomorphism
exact pair-vice dose differences for Bijvoet mates

http://skuld.bmsc.washington.edu/cgi-bin/MAD_power.pl

Resolution	RFriedel(%)	I/Sigma	Redundancy
10.12	0.8	74.1	23.7
6.90	0.8	43.6	23.7
5.34	1.1	48.4	23.0
4.51	1.2	47.5	23.5
3.98	1.6	34.5	20.6
3.60	2.5	22.4	13.9
3.31	4.0	14.0	11.9
3.08	6.6	8.3	7.0
2.89	10.5	5.2	6.1
2.73	15.6	3.7	2.5
2.60	23.0	2.4	3.8

Estimate of signal from MAD experiment

8 Se atoms per 300 protein residues and 0 nucleic acid residues

Se scattering factor estimates:

min f' = -9 max f' = -2 max f'' = 6

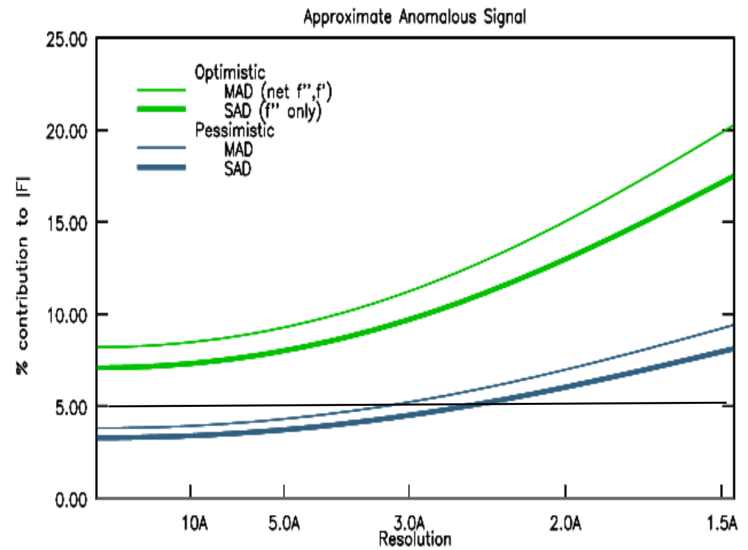
Pessimistic scenario:

60% of anomalous scatterers ordered; 60% of optimal f' and f'' achieved

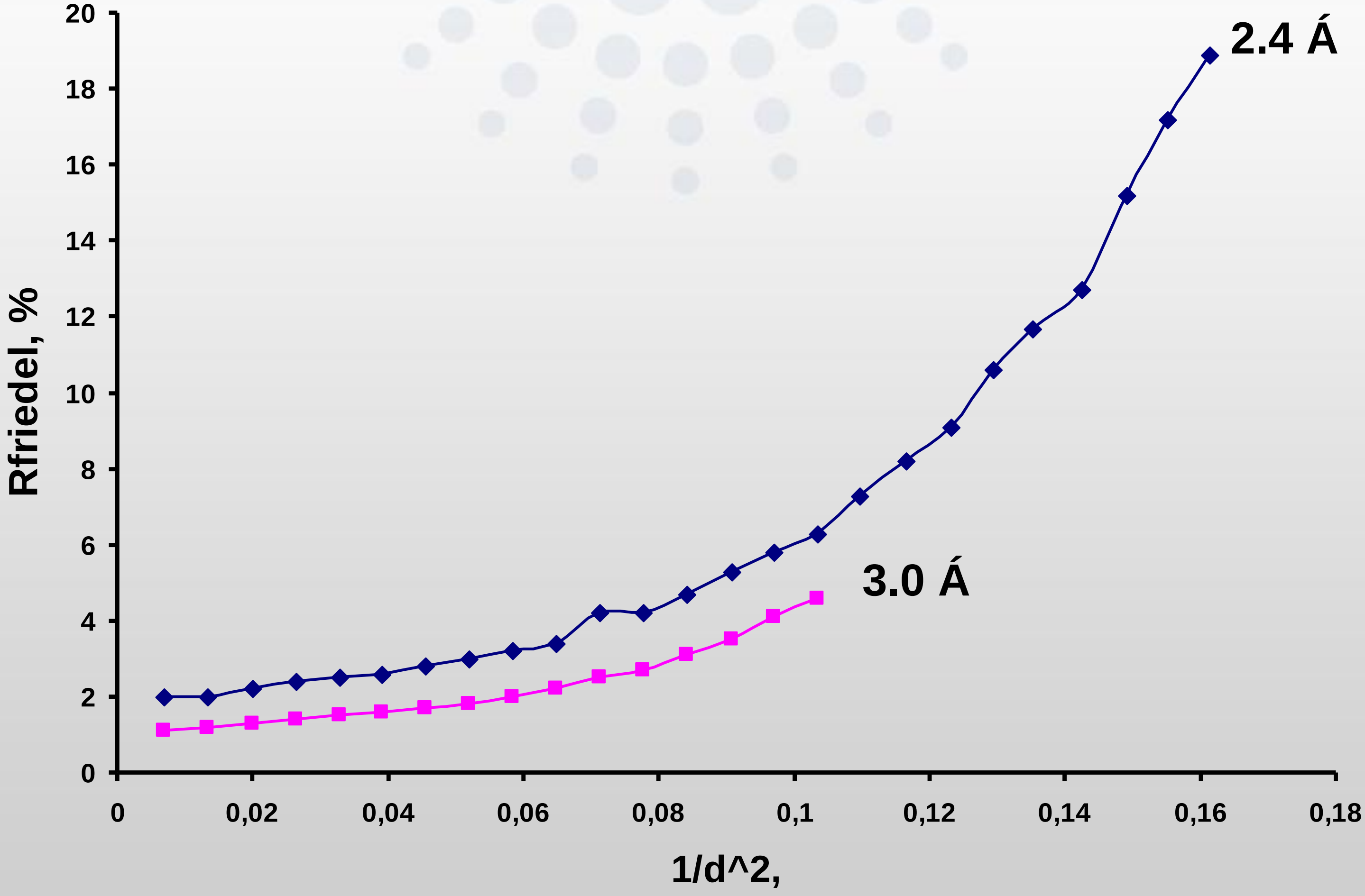
Resolution 6.011 Å

Signal 0.08392 %

Retrieve EPS version of plot



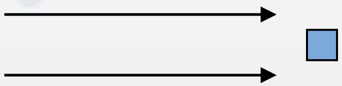
Rfriedel vs. resolution



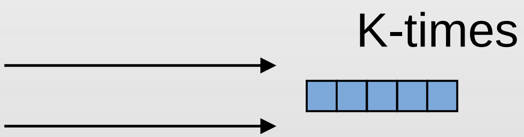
Multi-crystal and multi-positional strategy

$$\sigma\,(I_{peak}) = SQRT\,(I_{peak} + I_{background})$$

$$I_{peak} / \sigma(I_{peak}) = n$$

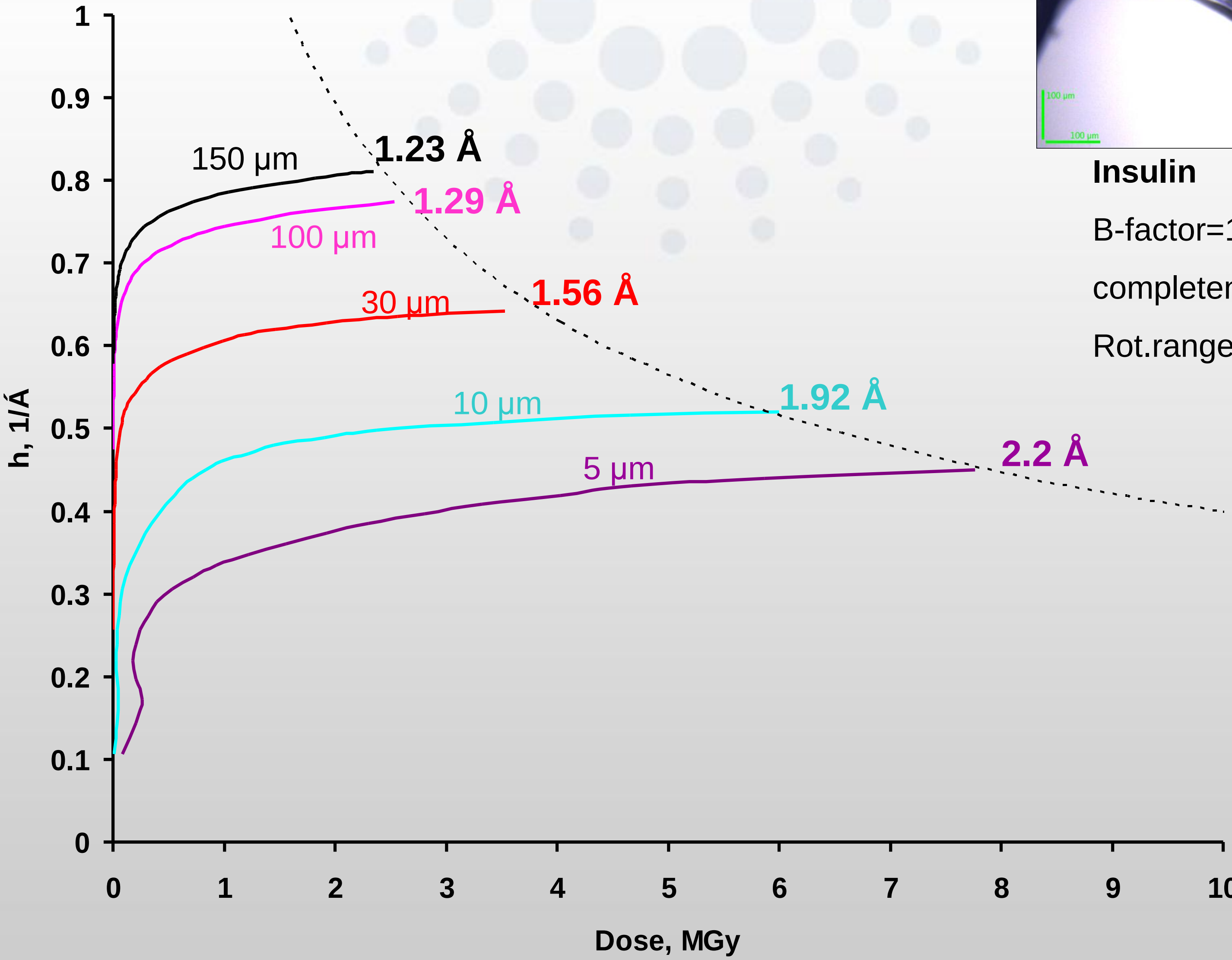


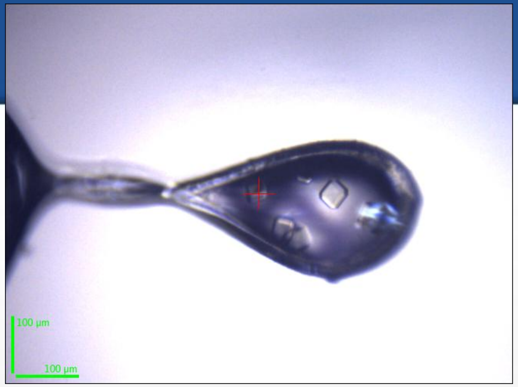
$$I_{peak} / \sigma(I_{peak}) = n * Sqrt(k)$$



Diffraction resolution vs. absorbed dose

for different crystal size





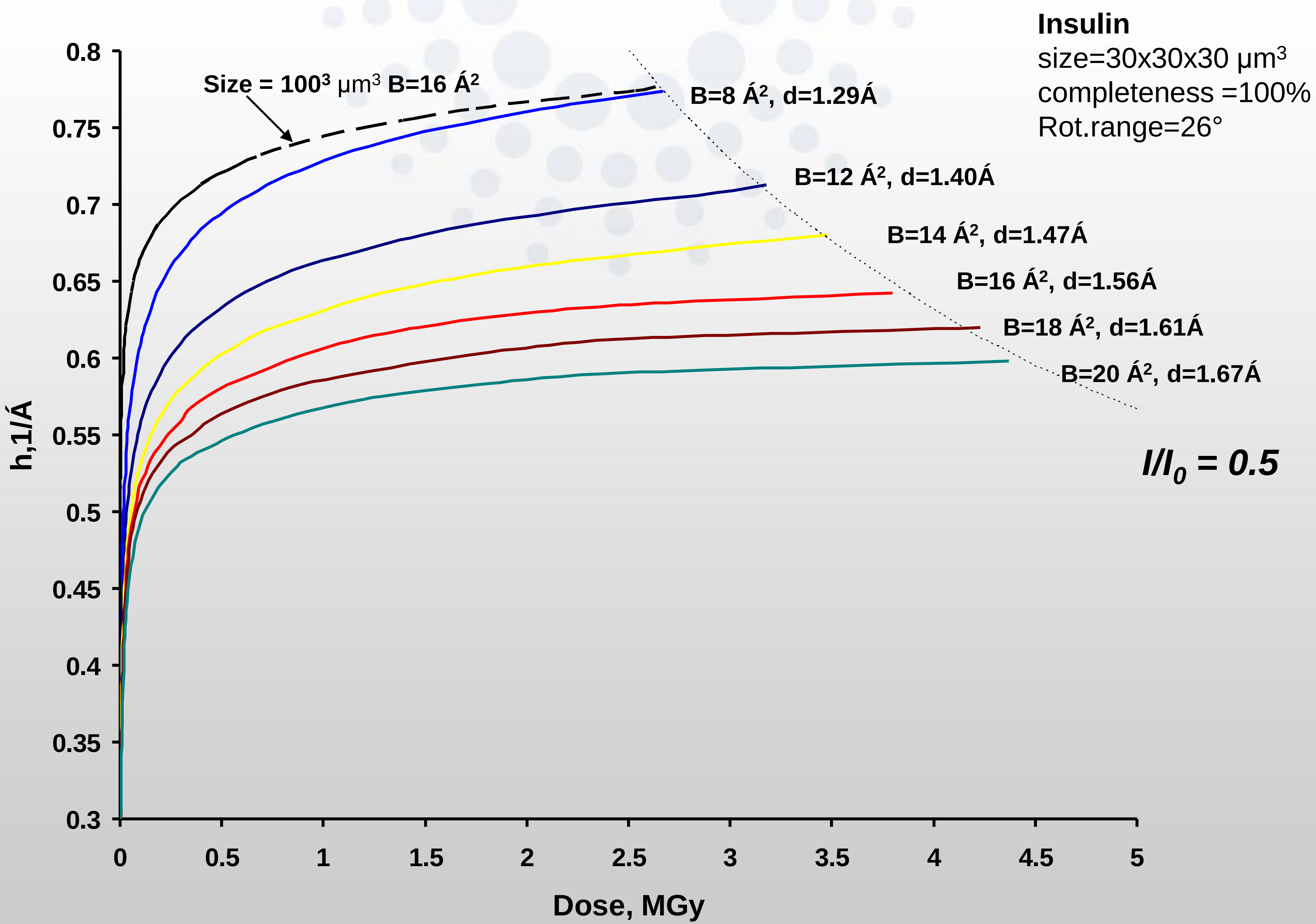
Insulin

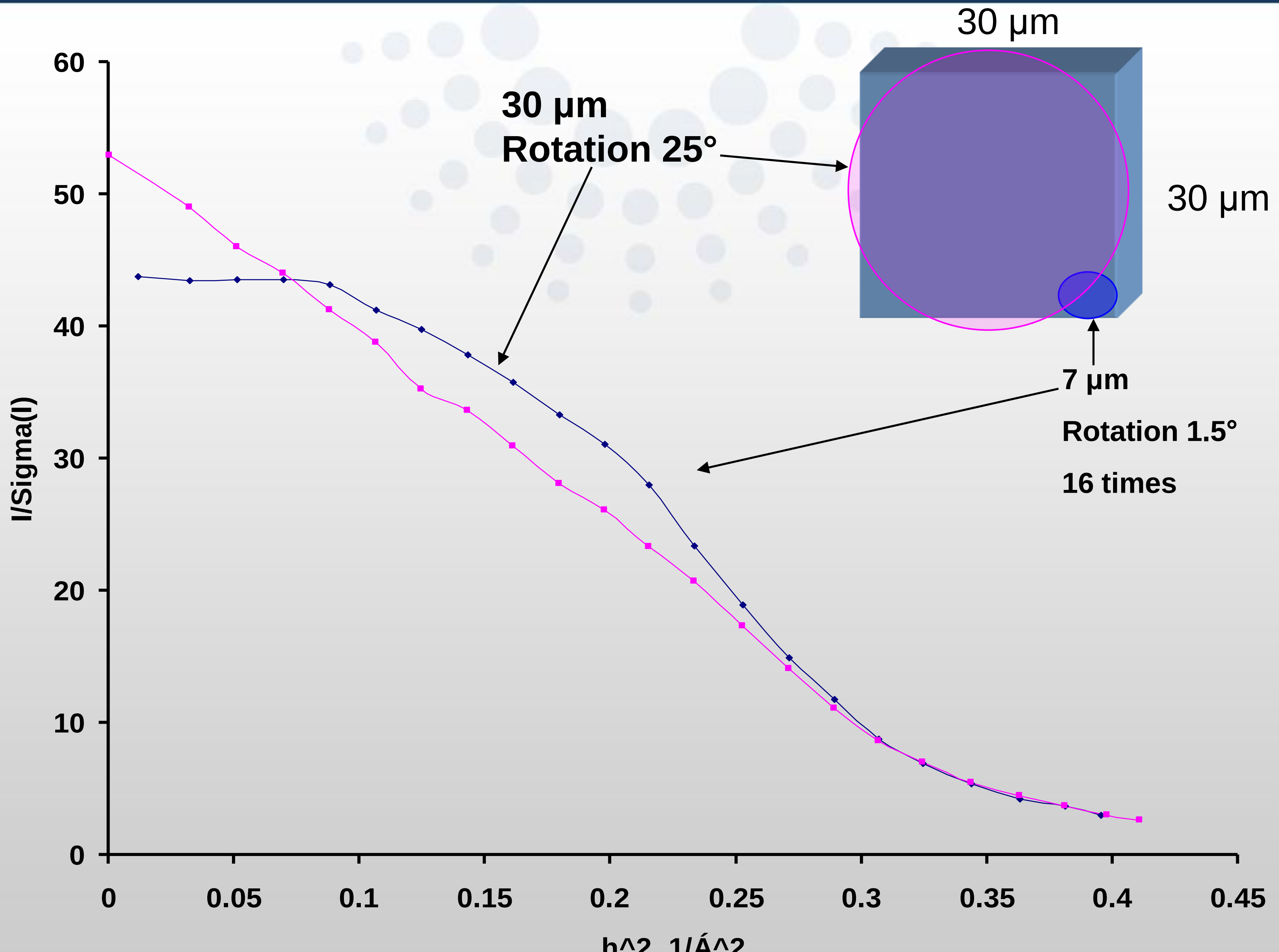
B-factor=16 Á^2

completeness =100%

Rot.range=26°

Diffraction resolution vs. absorbed dose
for different crystal B-factor

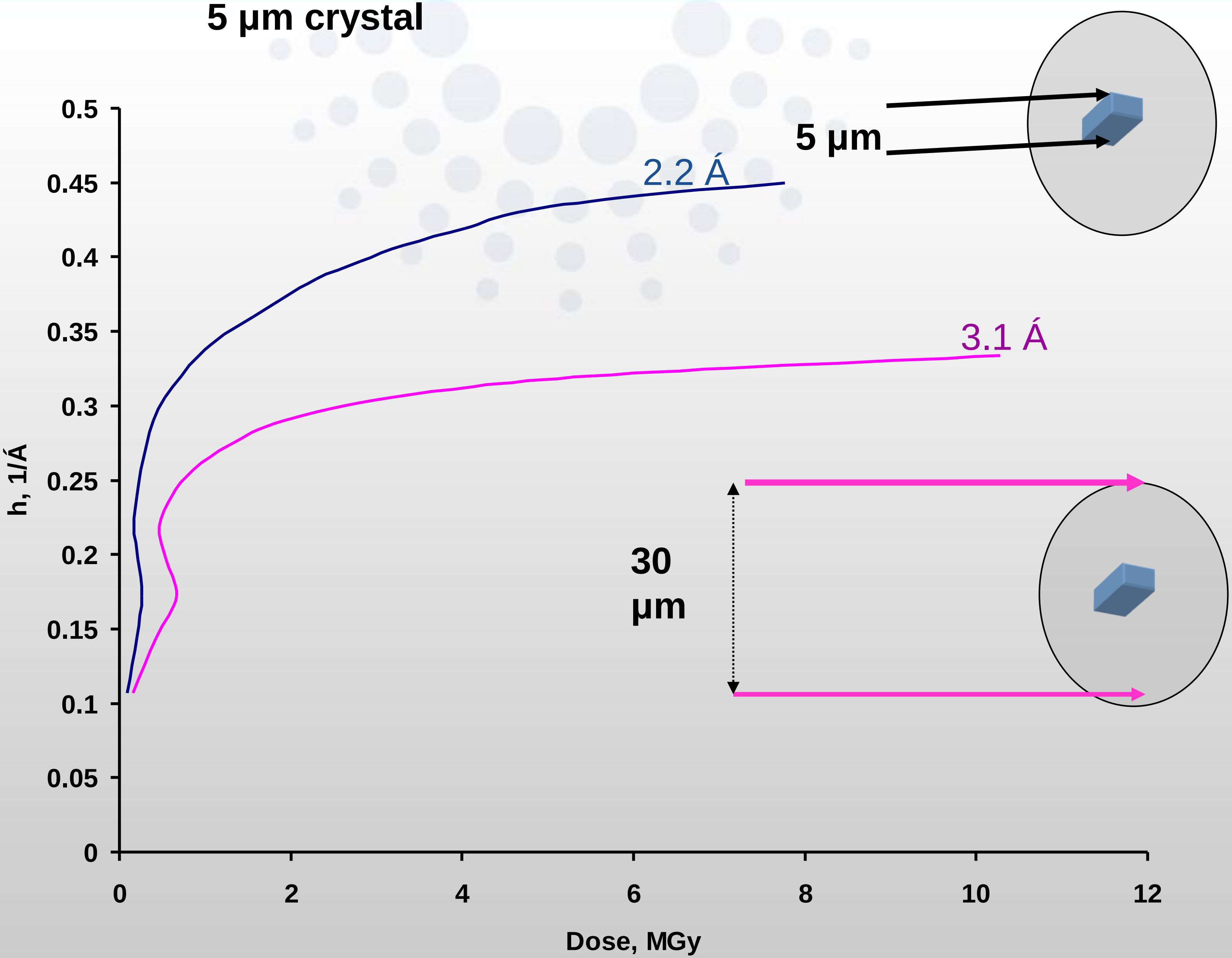




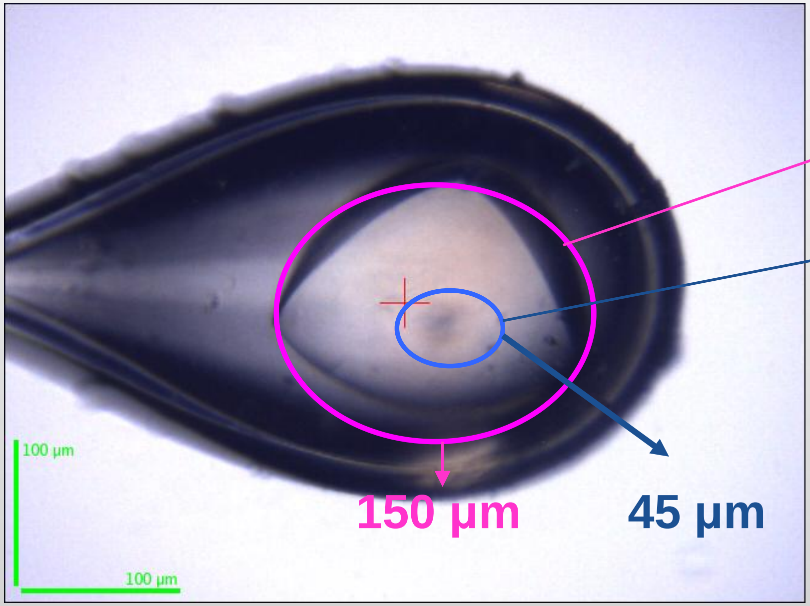
30 µm
30 µm
30 µm
Rotation 25°
7 µm
Rotation 1.5°
16 times
60
50
40
30
20
10
0
I/Sigma(I)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
h^2, 1/Á^2

Diffraction resolution vs. absorbed dose

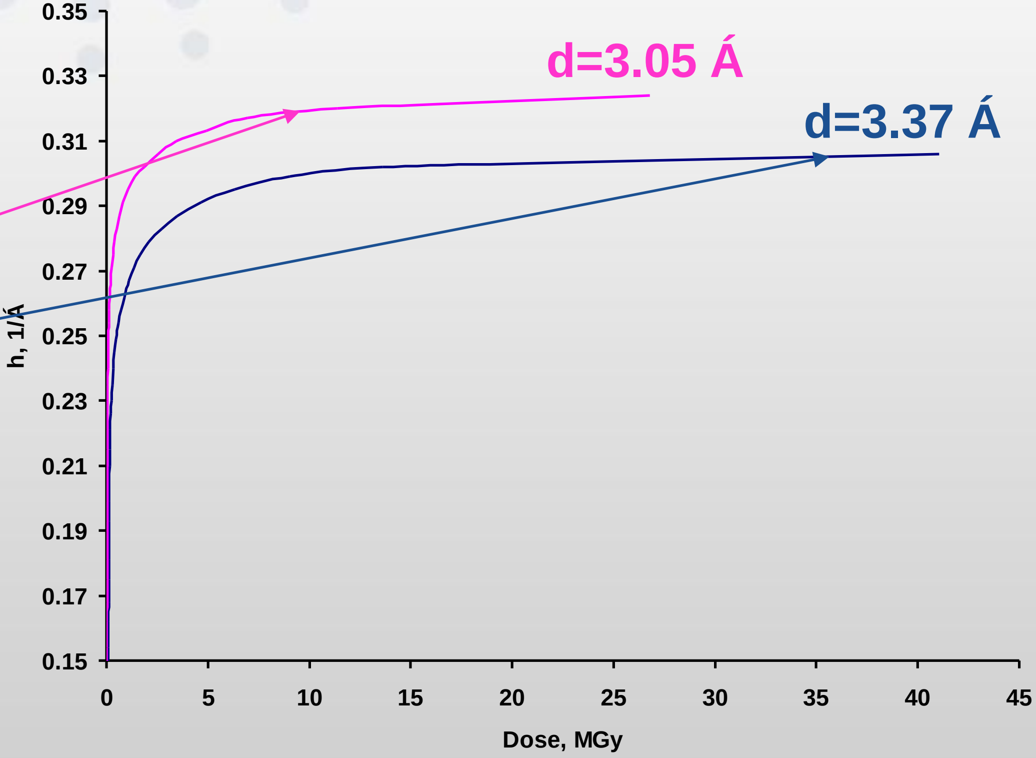
5 µm crystal



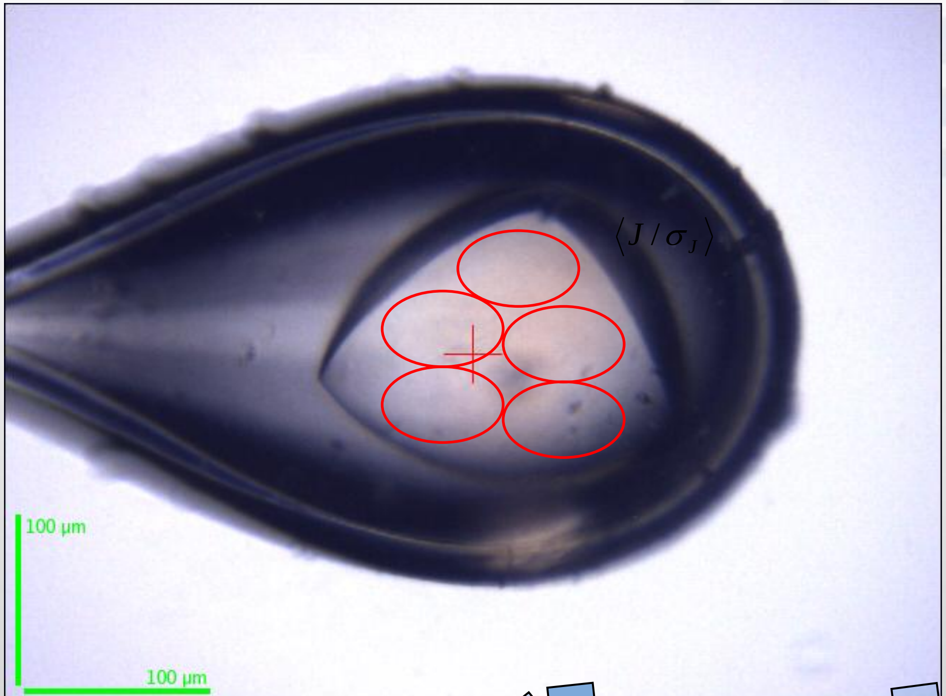
The 70 kDa membrane protein FtsH from Aquifex aeolicus
I222, a = 137.9, b = 162.1, c = 170

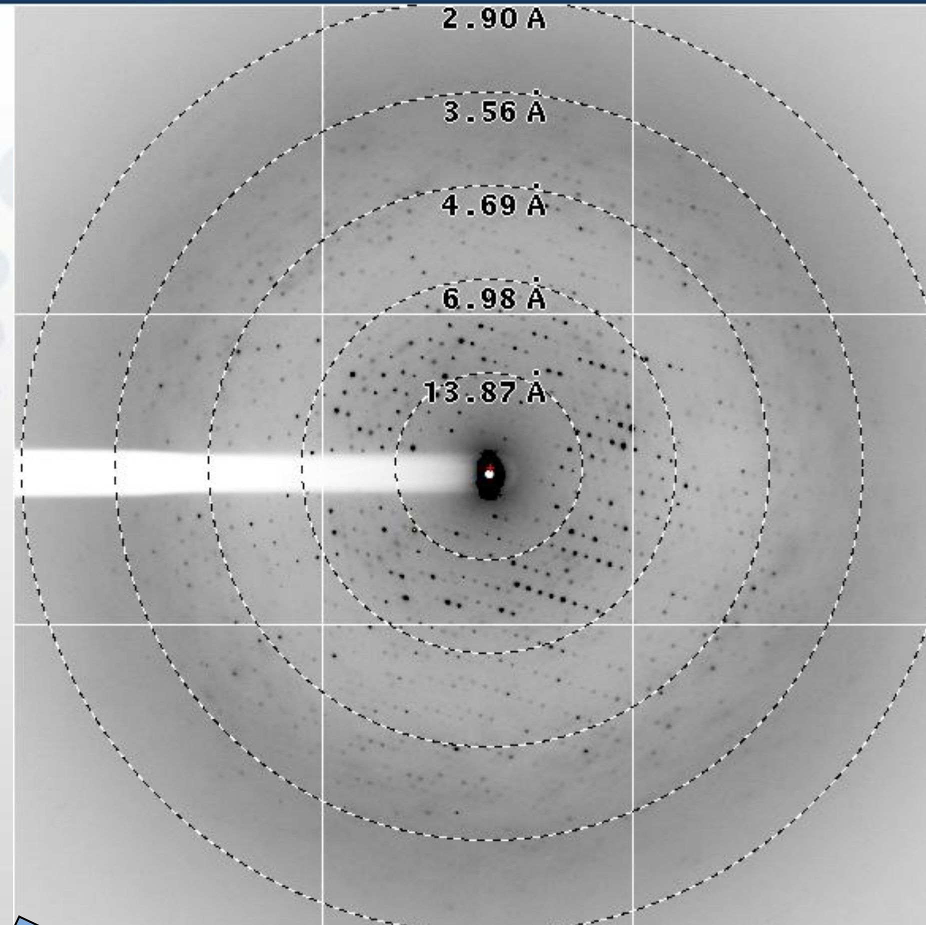


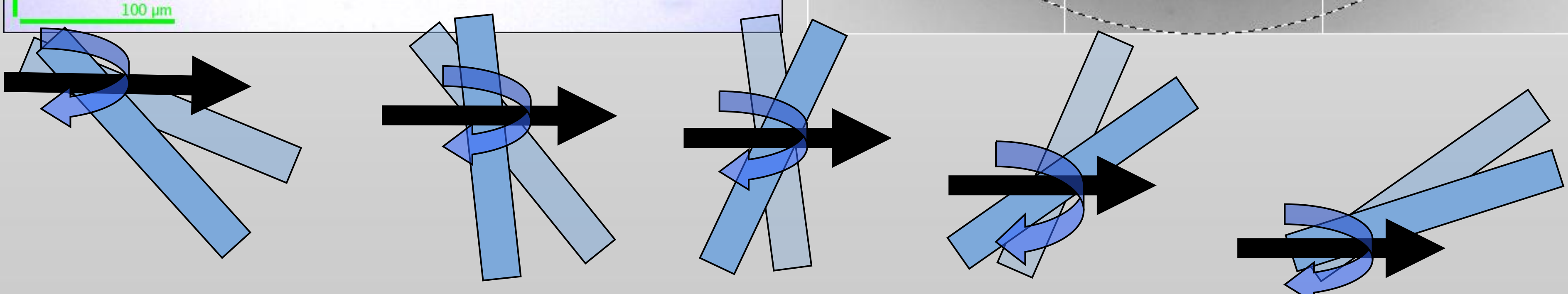
Diffraction resolution vs. absorbed dose



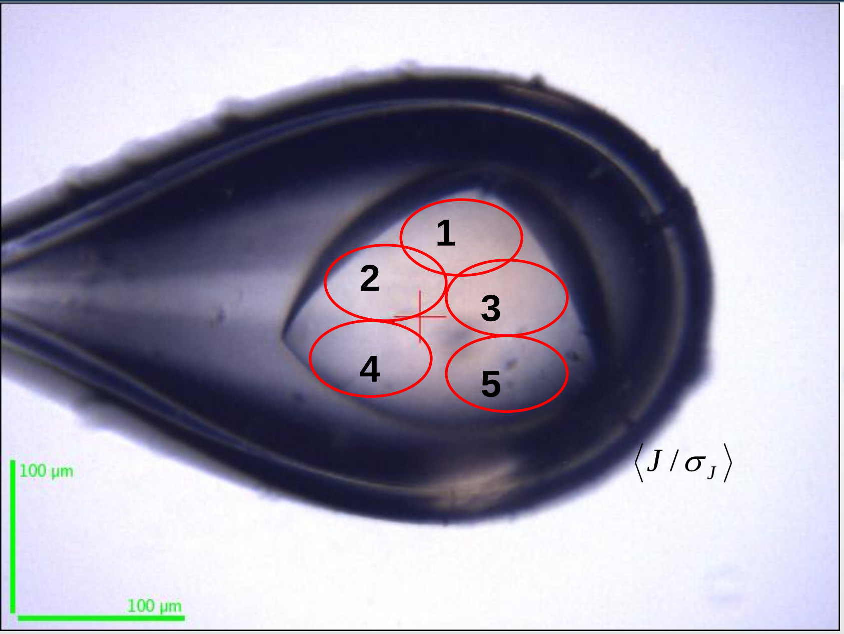
The 70 kDa membrane protein FtsH from Aquifex aeolicus
I222, a = 137.9, b = 162.1, c = 170



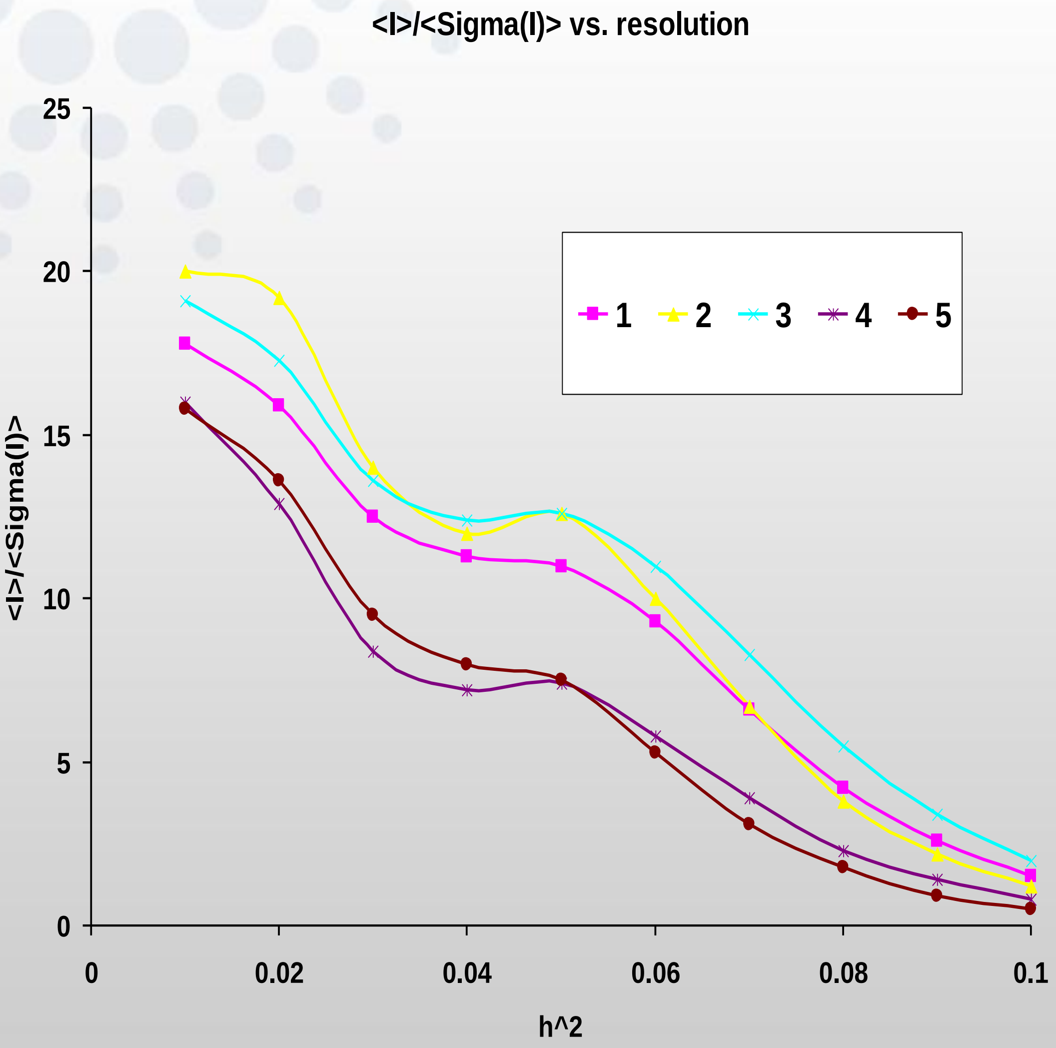




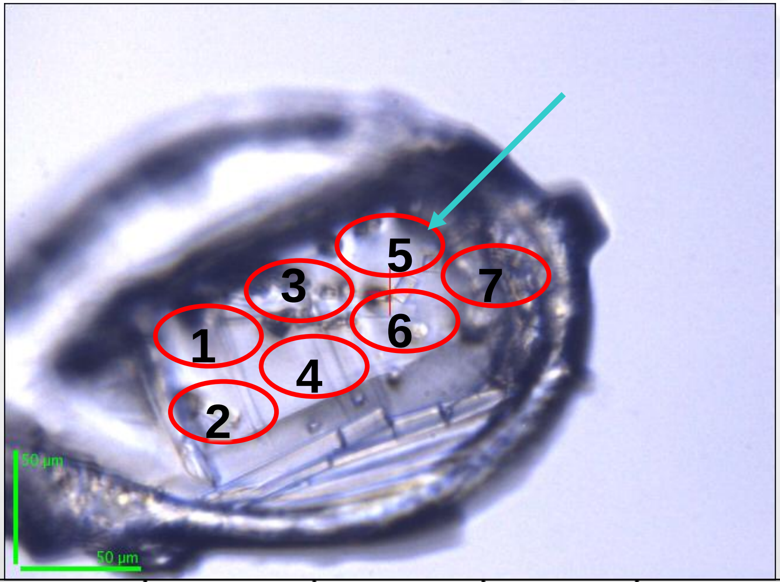
The 70 kDa membrane protein FtsH from Aquifex aeolicus
I222, a = 137.9, b = 162.1, c = 170

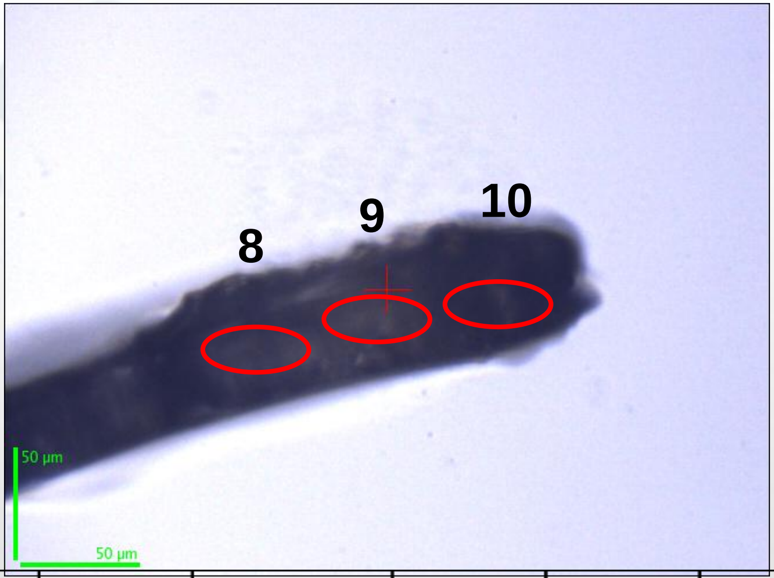


	B-factor	Scale
1	95	0.8
2	102	0.7
3	101	1
4	110	0.9
5	114	0.8

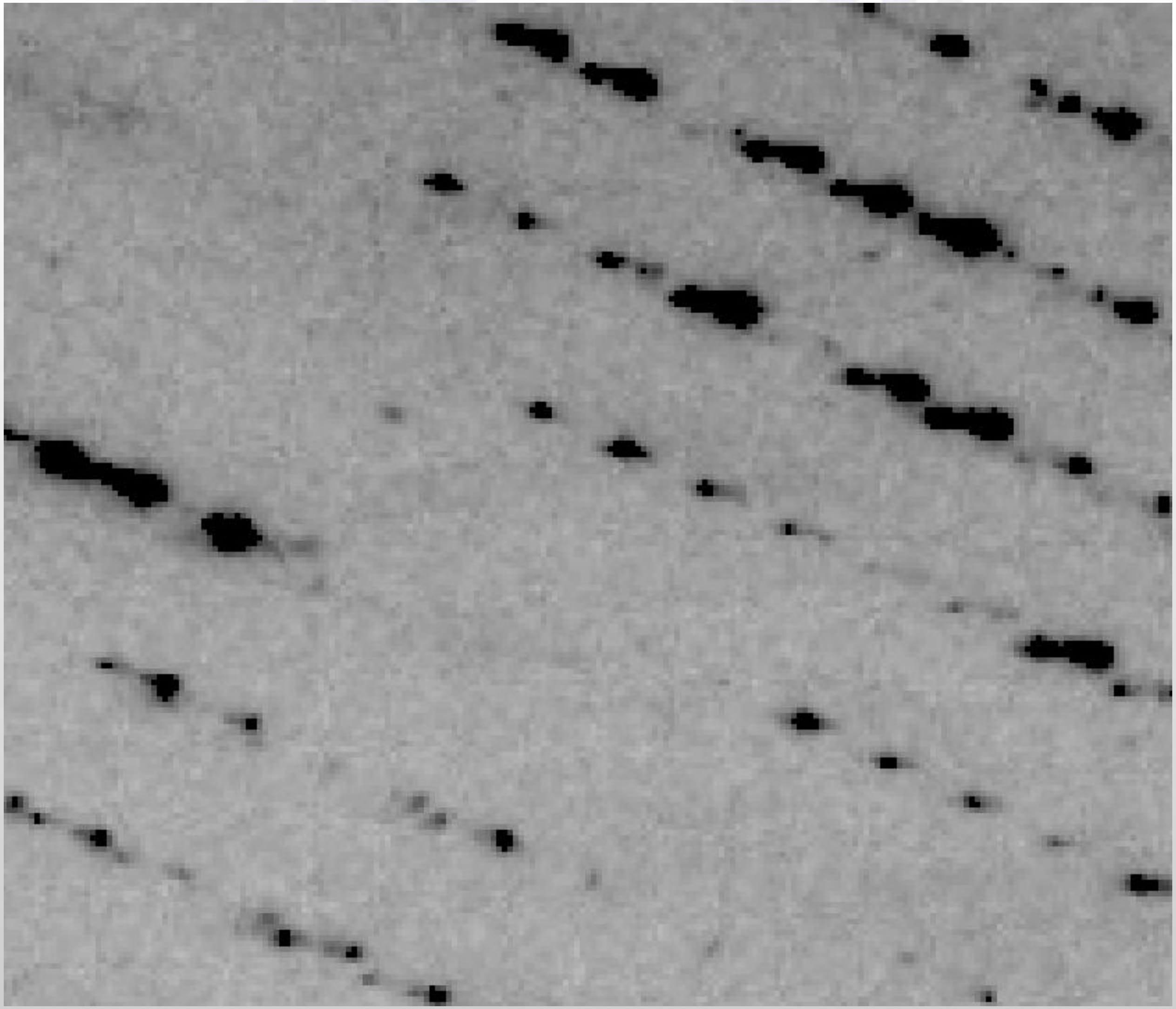


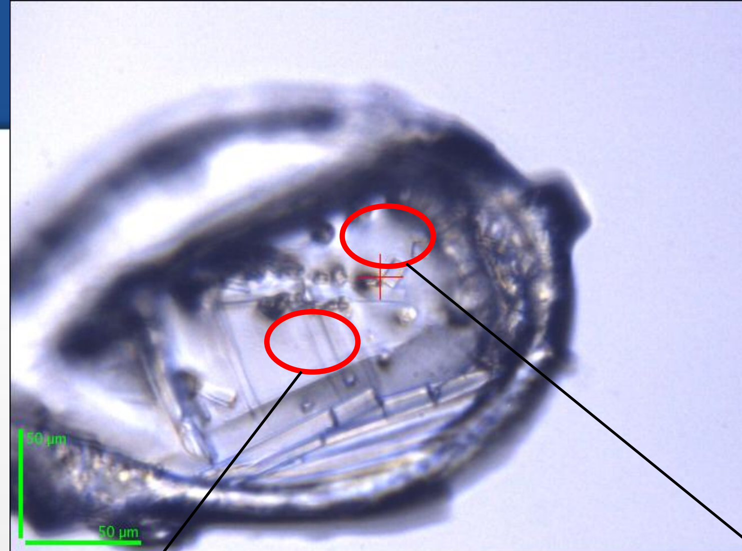
Scanning Diffraction
ID23-1 ESRF, Thermolysin P622

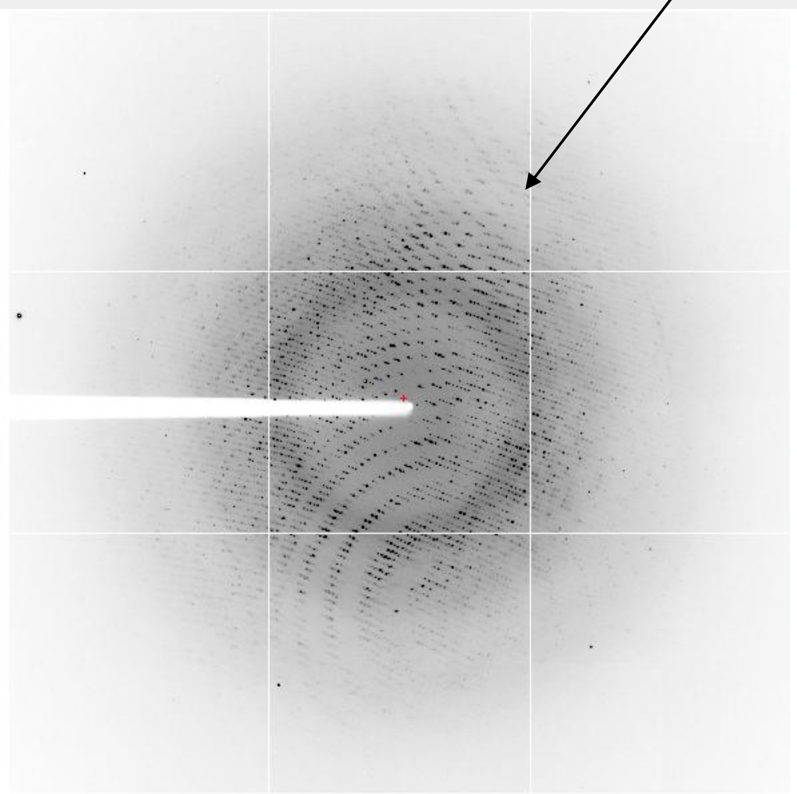


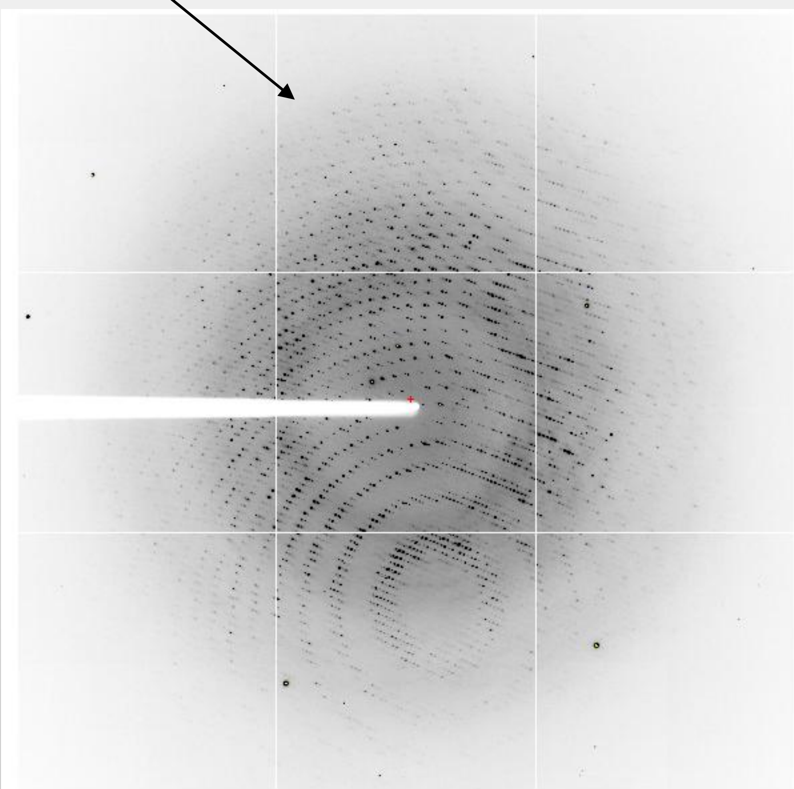


	1	2	3	4	5	6	7	8	9	10
B-factor	**18.3**	**15.6**	**23.7**	**20.9**	**13.0**	**19.4**	**21.7**	**16.4**	**20.9**	**19.5**
Scale	0.38	0.30	0.38	0.42	0.33	0.65	0.55	0.41	0.63	1.0
BEST D_{min} Å	1.85	1.90	2.15	2.01	1.74	1.83	1.98	1.96	2.01	1.85
mosaicity	0.42	0.19	0.26	0.40	0.38	0.50	0.54	0.46	0.56	0.48
a	93.8	93.7	93.4	93.5	93.9	93.8	93.6	93.8	93.7	93.8
c	140.3	140.5	140.5	139.9	140.1	139.8	139.8	140.5	140.1	140.0



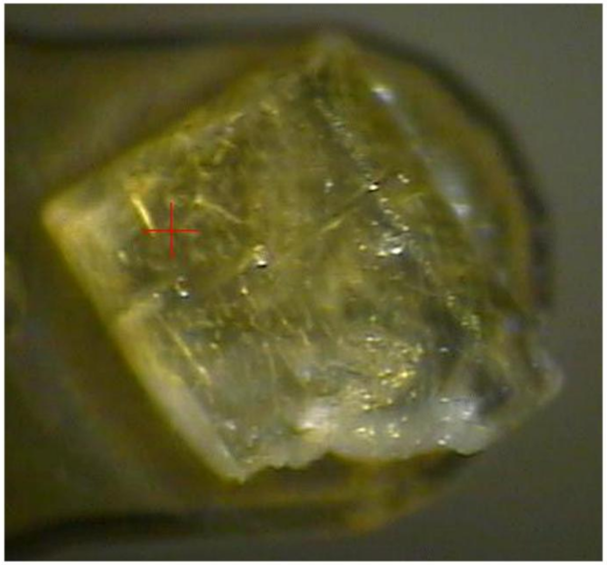

50 µm
50 µm

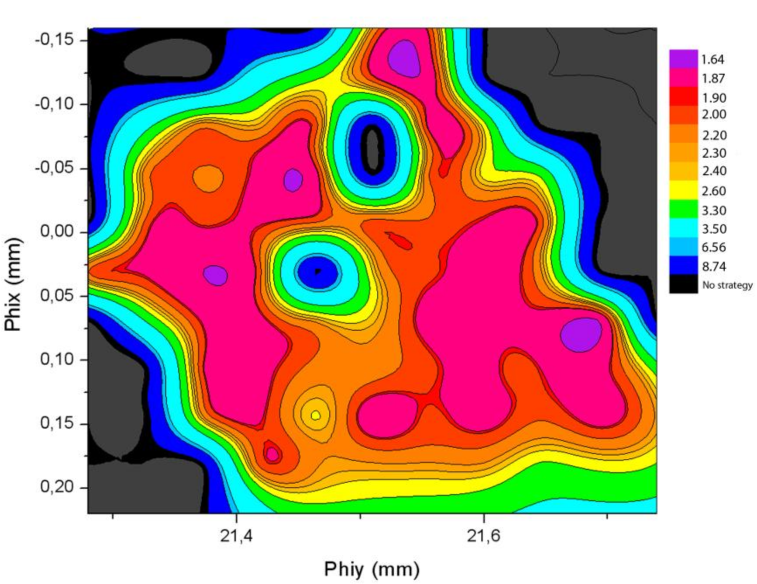




Diffraction Cartography

Matthew W. Bowler





Some conclusions

by Ruslan Sanishvili et al.

Acta Crystal. D, 2008; 64(Pt 4): 425–435.

- **The best quality diffraction data are obtained when the size of the X-ray beam is matched to the size of the sample crystal to the upper limit of the beam size**
- **Diffraction quality is reduced when the intercepted crystal volume inhomogeneous**
- **In practice, the length scale of many crystal inhomogeneities lies between 10 and 100 μm.**
- **Mini-beam should be used with small crystals to maximize diffraction quality.**
- **A mini-beam can be used routinely to probe large crystals for their most perfect regions. For more robust and streamlined operations, new tools are needed to automate this process.**

Multi-positional data collection tests *17/05/2011*

FAE crystals
ID23-1
E=12.75Kev
I=35 mA
Aperture=0.03 mm
Flux=1.5x10^{11} Photon/sec

FAE1 – 4 positions

FAE2 – 6 positions

FAE1 crystal

```
                        Multi-positions data collection
                       =================================
      Resolution limit is set by the radiation damage
Resolution limit =1.73 Angstrom   Transmission =  100.0%  Distance = 244.6mm
-----------------------------------------------------------------------------------------
          WEDGE PARAMETERS       ||                 INFORMATION
----------------------------------||-------------------------------------------------------
sub-| Phi  |Rot.  | Exposure| N.of||Over|sWedge|Exposure|Exposure| Dose   | Dose   |Comple-
 We-|start |width | /image  | ima-||-lap| width| /sWedge| total  |/sWedge| total  |teness
 dge|degree|degree|     s   |  ges||    |degree|   s    |   s    | MGy   |  MGy   |  %
----------------------------------||-------------------------------------------------------
   Wedge number =   1 Crystal position =  1
 1     0.00   0.25     1.338    80|| No   20.00    107.0    107.0   4.067   4.067   51.9
   Wedge number =   2 Crystal position =  2
 1    20.00   0.25     1.338    80|| No   20.00    107.0    107.0   4.067   4.067   85.6
   Wedge number =   3 Crystal position =  3
 1    40.00   0.25     1.338    80|| No   20.00    107.0    107.0   4.067   4.067   75.2
   Wedge number =   4 Crystal position =  4
 1    60.00   0.25     1.338    80|| No   20.00    107.0    107.0   4.067   4.067   88.1
-----------------------------------------------------------------------------------------

 Phi_start - Phi_finish      : 0.00 -  80.00
 Total rotation range        : 80.00 degree
 Total N.of images           : 320
 Overall Completeness        : 98.6%
 Redundancy                  : 3.18
 R-factor (outer shell)      : 5.6% (   36.8%)
 I/Sigma (outer shell)       : 22.9 (   3.3)
 Total Exposure time         : 428.1 sec (0.119 hour)
 Total Data Collection time  : 1228.1 sec (0.341 hour)
```

Wedge Data Collection Statistics according to the Strategy

Resolution Lower	Resolution Upper	Compl. %	Average Intensity	Average Sigma	<I>/<Sigma>	<I/Sigma>	R-fact %	Overload %
12.00	6.12	91.3	14005.4	423.9	32.1	33.8	3.3	0.00
6.12	4.64	95.6	12313.0	367.3	31.6	33.6	3.5	0.00
4.64	3.88	97.0	16992.6	500.7	31.0	33.1	3.7	0.00
3.88	3.41	97.3	12915.4	387.1	29.9	31.9	3.8	0.00
3.41	3.07	98.3	8411.2	262.1	28.5	30.5	4.1	0.00
3.07	2.82	98.4	5376.2	181.5	26.3	27.9	4.5	0.00
2.82	2.62	98.6	3759.6	142.3	23.5	24.9	5.0	0.00
2.62	2.46	98.4	2966.9	125.0	21.2	22.3	5.6	0.00
2.46	2.32	98.4	2495.5	118.4	18.9	19.9	6.3	0.00
2.32	2.21	99.0	2172.1	115.1	17.0	17.9	7.1	0.00
2.21	2.11	98.8	1821.0	112.9	14.7	15.5	8.2	0.00
2.11	2.02	99.1	1476.8	109.8	12.5	13.2	9.7	0.00
2.02	1.95	99.0	1107.6	106.4	9.9	10.4	12.3	0.00
1.95	1.88	99.4	797.1	104.3	7.4	7.8	16.5	0.00
1.88	1.81	99.6	585.8	106.3	5.4	5.7	22.4	0.00
1.81	1.76	99.8	441.0	108.5	4.0	4.2	30.1	0.00
1.76	1.73	100.0	370.8	111.3	3.3	3.5	36.8	0.00
All data		98.6	3687.0	161.3	21.3	17.0	5.6	0.00

R-fact = SUM (ABS(I - <I>)) / SUM (I)

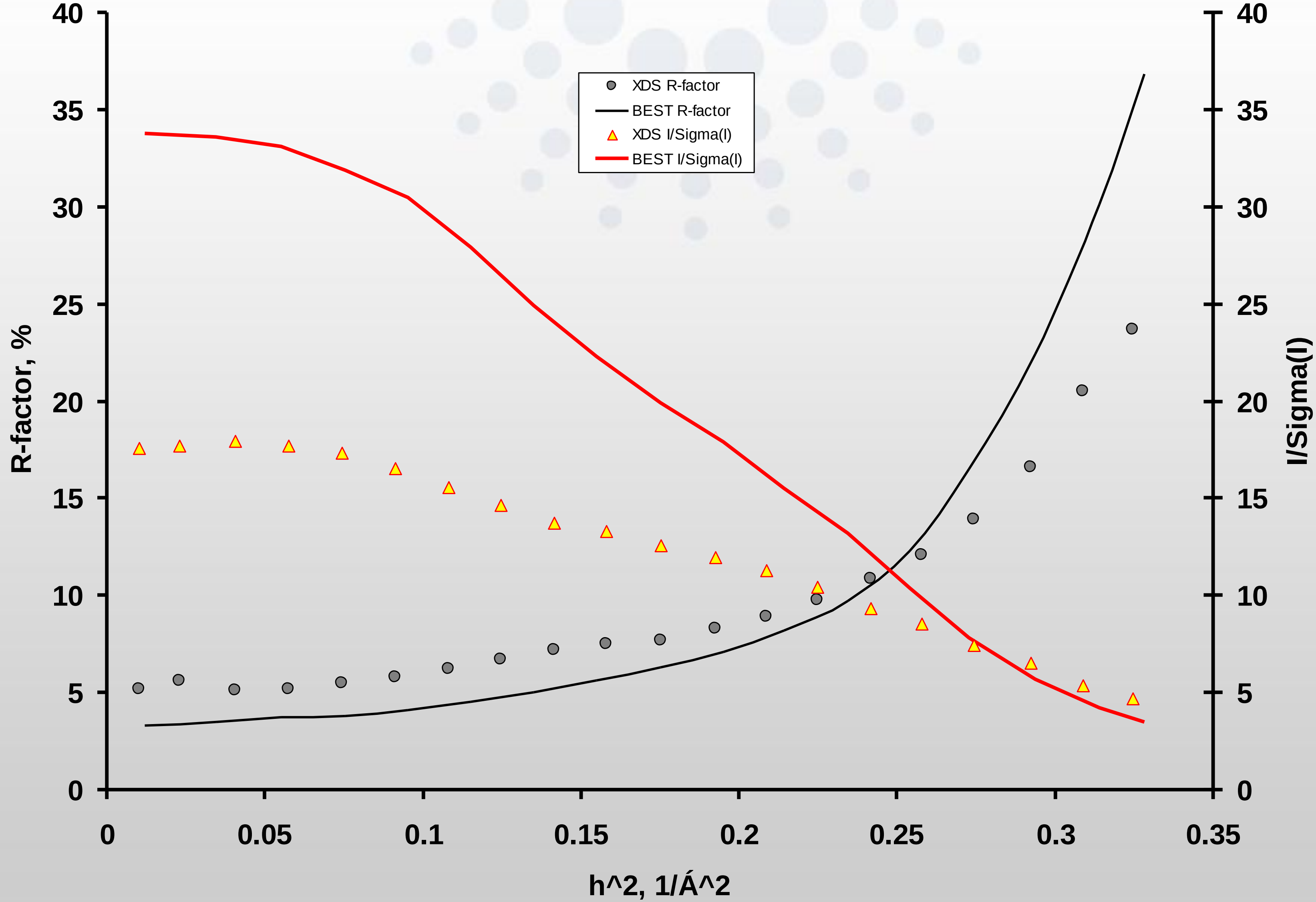
BEST predicted statistics

XSCALE

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

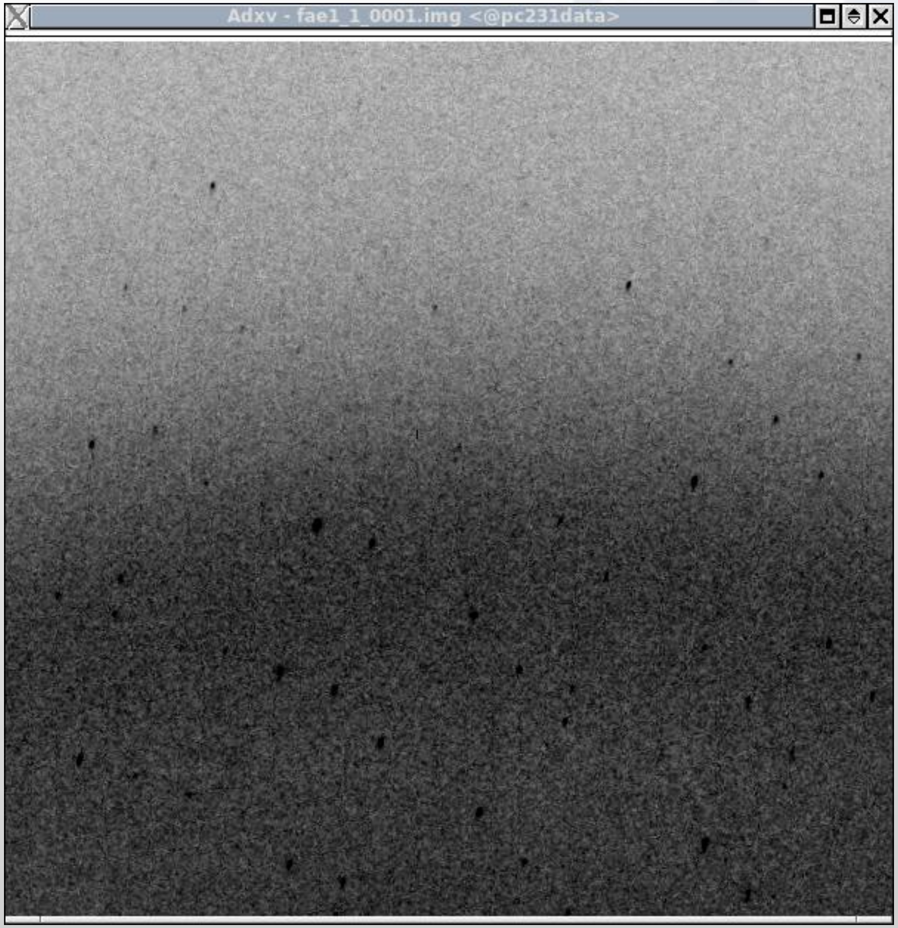
RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Anomal Corr	SigAno	Nano
7.74	2802	936	1099	85.2%	5.2%	6.1%	2692	17.56	6.2%	4.0%	51%	1.080	398
5.47	5493	1749	1845	94.8%	5.6%	6.2%	5313	17.68	6.7%	4.2%	59%	1.199	770
4.47	7119	2247	2361	95.2%	5.1%	6.2%	6882	17.97	6.0%	3.9%	41%	1.012	929
3.87	8125	2601	2710	96.0%	5.2%	6.2%	7858	17.71	6.2%	4.0%	34%	0.954	964
3.46	9500	2991	3093	96.7%	5.5%	6.2%	9237	17.32	6.5%	4.3%	36%	0.999	1183
3.16	10538	3302	3400	97.1%	5.8%	6.4%	10284	16.52	6.9%	4.7%	42%	1.044	1275
2.93	11461	3569	3674	97.1%	6.2%	6.6%	11214	15.55	7.4%	5.4%	37%	1.057	1390
2.74	12400	3827	3916	97.7%	6.7%	6.9%	12185	14.65	8.0%	5.7%	35%	1.045	1473
2.58	13227	4092	4186	97.8%	7.2%	7.3%	12993	13.71	8.6%	6.7%	39%	1.062	1519
2.45	14116	4320	4407	98.0%	7.5%	7.6%	13905	13.29	9.0%	6.8%	37%	1.044	1612
2.33	14784	4530	4620	98.1%	7.7%	8.0%	14557	12.56	9.2%	7.2%	29%	0.993	1684
2.23	15534	4758	4844	98.2%	8.3%	8.5%	15295	11.96	9.9%	8.1%	28%	0.993	1737
2.15	16105	4927	5001	98.5%	8.9%	9.0%	15857	11.32	10.7%	8.9%	26%	0.974	1789
2.07	16849	5152	5220	98.7%	9.8%	9.9%	16602	10.42	11.7%	10.0%	26%	0.969	1870
2.00	17478	5356	5407	99.1%	10.9%	11.4%	17191	9.36	13.0%	11.6%	22%	0.916	1953
1.94	17863	5504	5538	99.4%	12.1%	12.7%	17545	8.57	14.4%	13.1%	19%	0.880	1979
1.88	18615	5738	5765	99.5%	13.9%	15.1%	18258	7.48	16.6%	15.5%	16%	0.826	2058
1.82	19098	5896	5913	99.7%	16.6%	17.9%	18716	6.55	19.8%	18.5%	12%	0.806	2128
1.78	19508	6039	6052	99.8%	20.5%	22.6%	19110	5.37	24.4%	24.6%	10%	0.792	2189
1.73	18934	5969	6262	95.3%	23.7%	26.5%	18435	4.68	28.3%	28.8%	9%	0.766	2098
total	269549	83503	85313	97.9%	7.4%	8.0%	264129	11.11	8.8%	8.8%	28%	0.944	30998

FAE1 multi-positional data collection

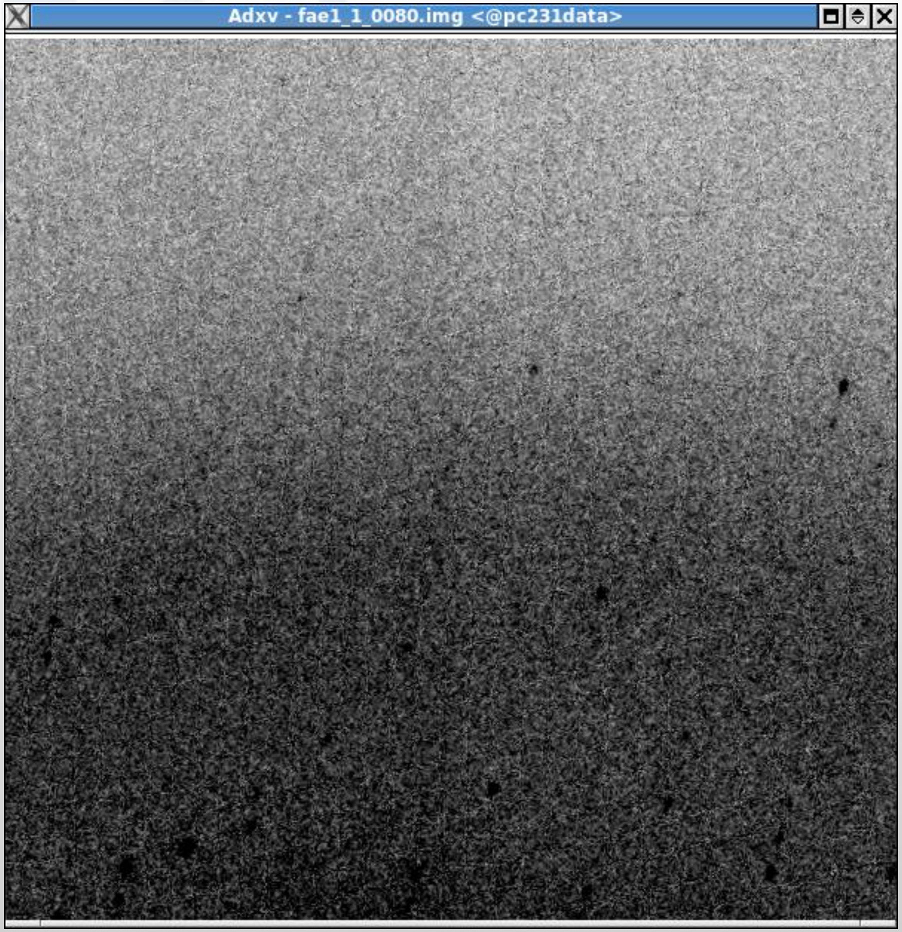


Radiation damage

Upper edge of two images



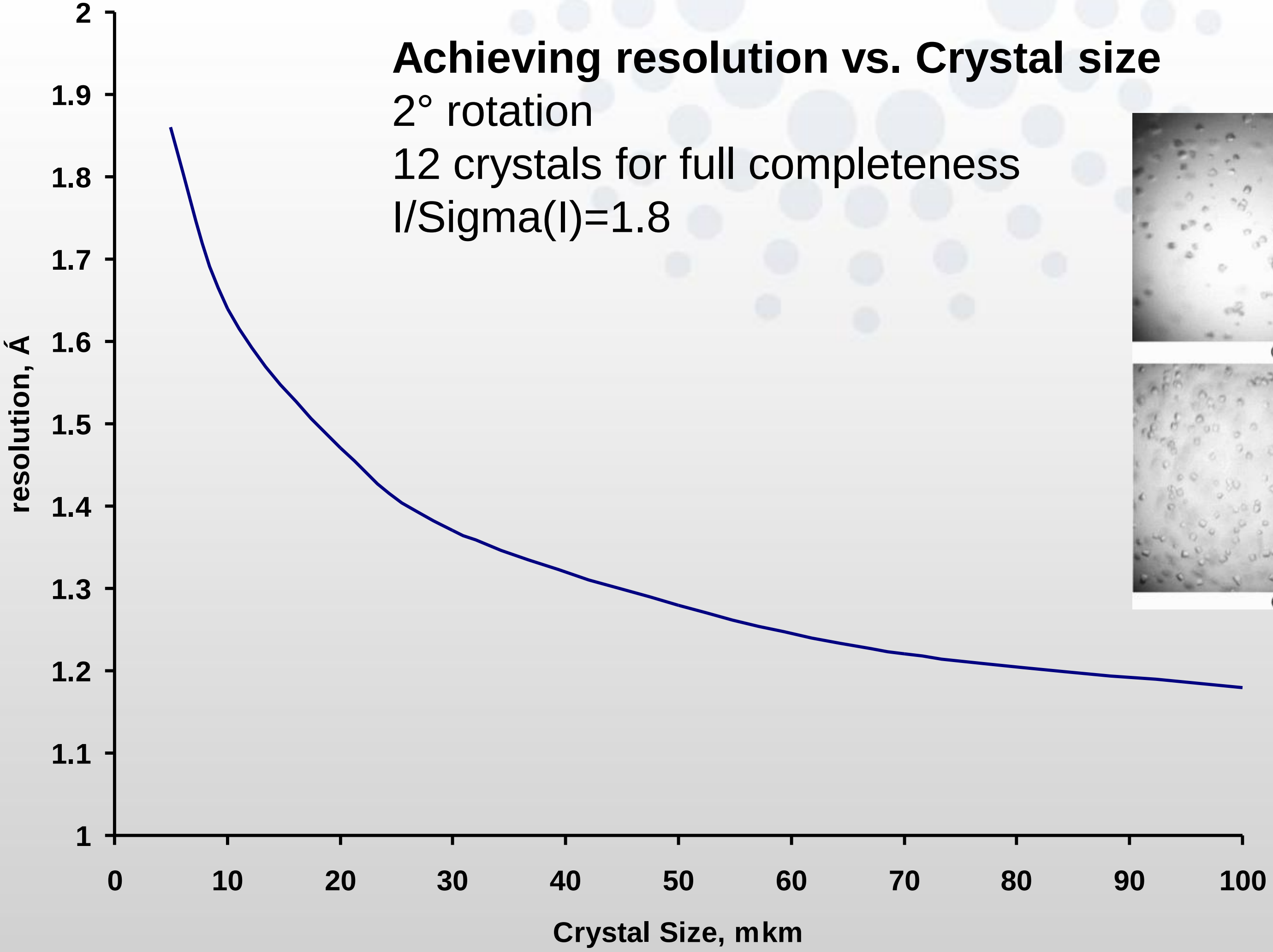
First image, position 1

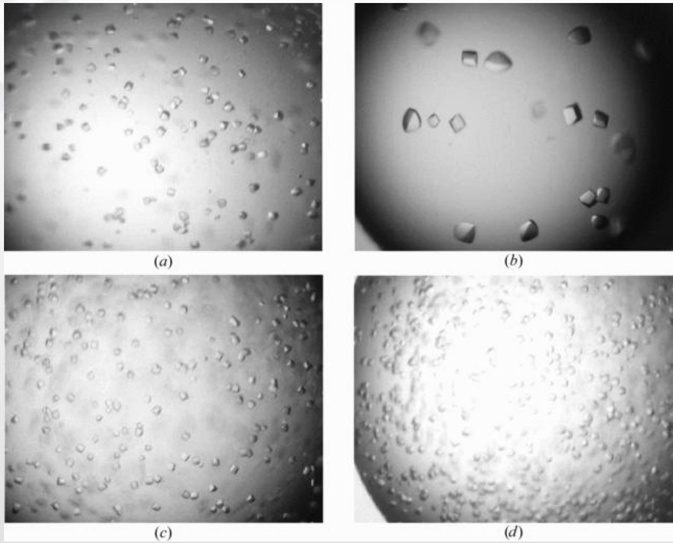


Last image, position 1

Multi-crystal data collection

Achieving resolution vs. Crystal size
2° rotation
12 crystals for full completeness
I/Sigma(I)=1.8





More Information

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